

**NOVEL POLYNUCLEOTIDES AND POLYPEPTIDES ENCODED THEREBY****RELATED APPLICATIONS**

This application claims priority to provisional patent applications USSN 60/156,745 filed September 30, 1999, USSN 60/158,942 filed October 6, 1999, 60/159,248 filed October 13, 1999, 60/169,344 filed December 6, 1999, and USSN 60/215,048 filed June 29, 2000 which are incorporated herein by reference in their entirety.

**FIELD OF THE INVENTION**

The invention relates in general to nucleic acids and polypeptides; more particularly it relates to polynucleotides expressed in the thymus gland and other tissues, and polypeptides encoded by such polynucleotides, as well as vectors, host cells, antibodies and recombinant methods for producing the polypeptides and polynucleotides.

**BACKGROUND OF THE INVENTION**

The invention relates generally to nucleic acids and polypeptides encoded thereby, and methods of using these nucleic acids and polypeptides.

**SUMMARY OF THE INVENTION**

The present invention is based in part on the discovery of novel polynucleotide sequences. These human nucleic acids and polypeptides encoded thereby are collectively referred to herein as "PTMAX".

Accordingly, in one aspect, the invention provides an isolated nucleic acid molecule that encodes a novel polypeptide, or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, e.g., a nucleic acid sequence encoding a polypeptide at least 85% identical to a

polypeptide comprising the amino acid sequences of SEQ ID NO:2n, wherein n is an integer between 1-10, or a polypeptide that is a fragment, homolog, analog or derivative thereof. The nucleic acid can include, e.g., one or more fragments from genomic DNA, or a cDNA molecule, or an RNA molecule. In particular embodiments, the nucleic acid molecule may include the sequence of any of SEQ ID NO:2n-1, wherein n is an integer between 1-10. These polypeptides and nucleic acids are related to a prothymosin alpha, an oncostatin or a nerve growth factor sequence, as disclosed herein.

Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

The invention is also directed to host cells transformed with a vector comprising any of the nucleic acid molecules described above.

In another aspect, the invention includes a pharmaceutical composition that includes a PTMAX nucleic acid and a pharmaceutically acceptable carrier or diluent.

In a further aspect, the invention includes a substantially purified PTMAX polypeptide, e.g., any of the PTMAX polypeptides encoded by a PTMAX nucleic acid, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition that includes a PTMAX polypeptide and a pharmaceutically acceptable carrier or diluent.

In a still further aspect, the invention provides an antibody that binds specifically to a PTMAX polypeptide. The antibody can be, e.g., a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition including PTMAX antibody and a pharmaceutically acceptable carrier or diluent. The invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

The invention also includes kits comprising any of the pharmaceutical compositions described above.

The invention further provides a method for producing a PTMAX polypeptide by providing a cell containing a PTMAX nucleic acid, e.g., a vector that includes a PTMAX nucleic

acid, and culturing the cell under conditions sufficient to express the PTMAX polypeptide encoded by the nucleic acid. The expressed PTMAX polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous PTMAX polypeptide. The cell can be, *e.g.*, a prokaryotic cell or eukaryotic cell.

5 The invention is also directed to methods of identifying a PTMAX polypeptide or nucleic acids in a sample by contacting the sample with a compound that specifically binds to the polypeptide or nucleic acid, and detecting complex formation, if present.

The invention further provides methods of identifying a compound that modulates the activity of a PTMAX polypeptide by contacting PTMAX polypeptide with a compound and  
10 determining whether the PTMAX polypeptide activity is modified.

The invention is also directed to compounds that modulate PTMAX polypeptide activity identified by contacting a PTMAX polypeptide with the compound and determining whether the compound modifies activity of the PTMAX polypeptide, binds to the PTMAX polypeptide, or binds to a nucleic acid molecule encoding a PTMAX polypeptide.

In another aspect, the invention provides a method of determining the presence of or predisposition of a PTMAX-associated disorder in a subject. The method includes providing a sample from the subject and measuring the amount of PTMAX polypeptide in the subject sample. The amount of PTMAX polypeptide in the subject sample is then compared to the amount of PTMAX polypeptide in a control sample. An alteration in the amount of PTMAX  
15 polypeptide in the subject protein sample relative to the amount of PTMAX polypeptide in the control protein sample indicates the subject has pathology related to a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder. A control sample is preferably taken from a matched individual, *i.e.*, an individual of similar age, sex, or other general condition but who is not suspected of having a dysfunction in the immune system, a  
20 tissue proliferation-associated condition, or a neurological disorder. Alternatively, the control sample may be taken from the subject at a time when the subject is not suspected of having a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder. In some embodiments, the PTMAX polypeptide is detected using a PTMAX antibody.

In a further aspect, the invention provides a method of determining the presence of, or predisposition to a PTMAX-associated disorder in a subject. The method includes providing a nucleic acid sample, *e.g.*, RNA or DNA, or both, from the subject and measuring the amount of the PTMAX nucleic acid in the subject nucleic acid sample. The amount of PTMAX nucleic acid sample in the subject nucleic acid is then compared to the amount of PTMAX nucleic acid in a control sample. An alteration in the amount of PTMAX nucleic acid in the sample relative to the amount of PTMAX in the control sample indicates the subject has a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder.

In a still further aspect, the invention provides a method of treating or preventing or delaying a PTMAX-associated disorder. The method includes administering to a subject in which such treatment or prevention or delay is desired a PTMAX nucleic acid, a PTMAX polypeptide, or a PTMAX antibody in an amount sufficient to treat, prevent, or delay an immune disorder, a tissue proliferation-associated disorder, or a neurological disorder in the subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

## DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel polypeptides and nucleotides encoded thereby. Included in the invention are ten novel nucleic acid sequences and their encoded polypeptides. The



sequences are collectively referred to as "PTMAX nucleic acids" or "PTMAX polynucleotides" and the corresponding encoded polypeptide is referred to as a "PTMAX polypeptide" or "PTMAX protein". For example, a PTMAX nucleic acid according to the invention is a nucleic acid including a PTMAX nucleic acid, and a PTMAX polypeptide according to the invention is a polypeptide that includes the amino acid sequence of a PTMAX polypeptide. Unless indicated otherwise, "PTMAX" is meant to refer to any of the novel sequences disclosed herein.

Table 1 provides a summary of the PTMAX nucleic acids and their encoded polypeptides.

Column 1 of Table 1, entitled "PTMAX No.", denotes a PTMAX number assigned to a nucleic acid according to the invention.

Column 2 of Table 1, entitled "Clone Identification Number" provides a second identification number for the indicated PTMAX.

Column 3 of Table 1, entitled "Tissue of Origin of the Clone", indicates the tissue in which the indicated PTMAX nucleic acid is expressed.

Columns 4-9 of Table 1 describe structural information as indicated for the indicated PTMAX nucleic acids and polypeptides.

Column 10 of Table 1, entitled "Protein Similarity" lists previously described proteins that are related to polypeptides encoded by the indicated PTMAX. Genbank identifiers for the previously described proteins are provided. These can be retrieved from <http://www.ncbi.nlm.nih.gov/>.

Column 11 of Table 1, entitled "Signal Peptide Cleavage Site" indicates the putative nucleotide position where the signal peptide is cleaved as determined by SignalP.

Column 12 of Table 1, entitled "Cellular Localization" indicates the putative cellular localization of the indicated PTMAX polypeptides.

Table 1.

PTMAX No.	Clone Identification Number	Tissue of Origin of the Clone	Nucleotide Length	Open Reading Frame (nt)	AA Residues	Calculated Molecular Weight	Protein Similarity	Signal Peptide Cleavage Site (nt)	Cellular Localization
1	AC009485_A	Genomic	327	1-327	109	11909.9	Pnr:REMTREMBL-ACC:G190372, human prothymosin alpha pseudogene;Pnr:SPTREMBL-ACC:Q15249, human prothymosin alpha	None	Cytoplasm
2	AC010175_A.0.1	Genomic, placenta, spleen	555	1-342	114	12389.2	ACC:AAA36485, human prothymosin-alpha pseudogene;	None	Cytoplasm
3	AC010175_A.9.5	Genomic, placenta, spleen	675	55-397	114	12481.4	REMTREMBL-ACC:AAA36485, human prothymosin-alpha pseudogene	None	Nucleus
4	AC009533_A	Genomic	345	1-342	114	12390.2	Pnr:REMTREMBL-ACC:G190372, human prothymosin alpha	None	Cytoplasm

								pseudogene;Ptr:SPTRE MBL-ACC:Q15249, human prothymosin alpha			
5	AL121585_A	Genomic	501	134-460	109	12005.8	ACC:g625274, prothymosin alpha - human; ACC:g135833, prothymosin alpha - bovine	None	Cytoplasm		
6	AC010175	Genomic	342	1-342	114	12389.2	Human prothymosin alpha	None	Cytoplasm		
7	AC010784-1	Genomic	324	1-324	108	11680.7	Oncostatin A (Platelet Factor 4 precursor)	Betw. Residues 40 and 41: AEA- EE	plasma membrane		
8	AL049825	Genomic	738	13-735	241	26958.5	Nerve Growth Factor		Extracellular or lysosome (lumen)		
9	AL121585_da1	Genomic	345	10-339	110	12071.8	Prothymosin alpha	None	Cytoplasm		
10	AL121585_da2	Genomic	350	10-348	113	12348.2	Prothymosin alpha	None	Cytoplasm		

Table 2 provides a cross reference to the assigned PTMAX number, clone identification number and sequence identification numbers (SEQ ID NOs.).

**Table 2.**

PTMAX No.	Clone Identification Number	SEQ ID NO Nucleic Acid	SEQ ID NO Polypeptide
1	AC009485_A	1	2
2	AC010175_A.0.1	3	4
3	AC010175_A.9.5	5	6
4	AC009533_A	7	8
5	AL121585_A	9	10
6	AC010175	11	12
7	AC010784-1	13	14
8	AL049825	15	16
9	AL121585_da1	17	18
10	AL121585_da2	19	20

5

PTMAX nucleic acids, and their encoded polypeptides, according to the invention are useful in a variety of applications and contexts. The various PTMAX nucleic acids and polypeptides according to the invention are useful, *inter alia*, as novel members of the protein families according to the presence of domains and sequence relatedness to previously described proteins.

10

For example, the PTMA1-6, 9 and 10 nucleic acids and their encoded polypeptides include structural motifs that are characteristic of proteins belonging to the prothymosin alpha family of proteins. Prothymosin alpha is a thymic hormone that has immunomodulatory, hematopoietic, and anti-neoplastic activities. In particular, prothymosin alpha has the same quantitative and qualitative biological activity as thymosin alpha; i.e., it has efficacy for treatment of immunodeficiency diseases, immunodepressed cancer patients, and for prevention of opportunistic infections in immunosuppressed patients. Thus, PTMA 1-6, 9 and 10 nucleic acids and polypeptides, antibodies and related compounds according to the invention will be useful in therapeutic applications implicated in various cancers and immunodeficiency disorders, *e.g.*, AIDS, autoimmune diseases, *e.g.*, lupus erythematosus and rheumatoid arthritis.

A peptide containing 28 amino acid residues, named thymosin-alpha-1, was originally isolated from calf thymosin fraction 5 and shown to restore various aspects of immune function in several in vitro and in vivo test systems. Thymosin-alpha-1 is one of several hormones or hormone-like substances produced by the thymus gland and derived from a polypeptide precursor. In 1984 Haritos et al. isolated a larger polypeptide precursor containing 113 amino acids from fresh rat thymus named prothymosin-alpha, which contains the thymosin-alpha-1 sequence at its NH2 terminus.

Thymosin-alpha-1 was subsequently isolated from a similar fraction from human thymus and reported to have the same amino acid sequence as bovine thymosin-alpha-1. Prothymosin alpha isolated from human thymus appears to represent the native polypeptide from which thymosin alpha 1, thymosin alpha 11 and other fragments are generated during isolation of thymosin fraction 5. Human prothymosin alpha is a polypeptide of 109 to 114 amino acid residues, and contains the entire thymosin alpha 1 sequence at its amino terminal. The peptide participates in the regulation, differentiation and function of thymic dependent lymphocytes and appears to be at least as potent on a weight basis as thymosin alpha1 in the protection of subject animals against opportunistic infections.

In general, the prothymosin alpha-like proteins of the present invention are thought to have the comparable quantitative and qualitative biological activity as thymosin alpha. An

anticipated dosage range is likely to be about 1-100 :g/kg/day. Dosages of the nucleic acids of the invention used in gene therapeutic applications are likely to be lower, and administration is likely to be less frequent, than the dosages shown for the proteins.

Human peripheral blood monocytes incubated with prothymosin alpha release thymosin alpha 1 in the culture supernatants. In addition total RNA is found to increase. The production of thymosin alpha 1 involves de novo protein synthesis as shown by the kinetics of its release and the inhibition of its synthesis by actinomycin D and cycloheximide. Thymosin alpha 1 release, possibly in association with HLA-DR, stimulates the proliferation of the T cell population.

Eckert et al. (Int J Immunopharmacol 1997 Sep-Oct;19(9-10):493-500) conducted preclinical studies with prothymosin alpha 1 on mononuclear cells from tumor patients. They studied the immunomodulating potential of the thymic protein, prothymosin alpha1 (Pro alpha1), on the lymphocyte and monocyte directed antitumor reactions of melanoma and colorectal tumors in cancer patients as compared to healthy controls. On average, they found that tumor patients showed lower NK-and LAK-cell activities than healthy controls, being associated with a lower adhesion capacity to tumor target cells. The NK-cell activity of the tumor patients was inversely related to the tumor stage. Pro alpha1 stimulated the impaired patients, LAK-cell activity only at an early stage of disease. The Pro alpha1 effects were associated with an increased adhesion of lymphocytes to tumor target cells and an increased secretion of deficient IFN-gamma and IL-2 secretion. By flow cytometry, Eckert et al. found that pro alpha1 in combination with IL-2 increased the NK-cell markers CD56, CD16/56 and CD25 as well as CD18/11a adhesion molecule expression. Monocytes from tumor patients showed deranged tumoristatic activities compared with healthy controls. Pro alpha1 elevated the mean of the antitumor activity, when applied alone or in combination with rIFN-gamma. In the presence of IFN-gamma, Pro alpha1 stimulated the adhesion of monocytes to cultured tumor cells, mainly by increasing CD54 expression. Pro alpha1 stimulated alone or in combination with IFN-gamma the TNF-alpha and IL-1beta secretion by monocytes and decreased the high PGE2 and TGF-beta level, especially in the test patient groups.

In addition, prothymosin alpha has been shown to increase the efficacy of anti-viral and chemotherapeutic agents. Accordingly, PTMA 1-6, 9 and 10 nucleic acids, polypeptides, antibodies and related compounds of the invention may be used to treat viral diseases such as hepatitis C as well as various malignancies. Furthermore, prothymosin alpha has been detected as a product of neoplastically transformed cells. PTMA 1-6, 9 and 10 nucleic acids and polypeptides, antibodies and related compounds according to the invention may have therapeutic and diagnostic applications as a diagnostic marker for cancer. Tissue expression analysis as described in EXAMPLE 2 below demonstrates the high expression PTMAX nucleic acids in various cancers, e.g., melanoma, colon and breast, suggesting a potential therapeutic applications of PTMAX nucleic acids and polypeptides either as a diagnostic marker for these cancers or in the treatment of these cancers.

PTMA 7, nucleic acid and encoded polypeptide includes structural motifs that are characteristic of proteins belonging to the oncostatin family of proteins. Oncostatin is an angiostatic CXC cytokine. Angiogenesis is an important normal physiologic process in embryogenesis, wound repair and the female reproductive cycle. However, as a pathological process, it plays a central role in chronic inflammation, fibroproliferative disorders and tumorigenesis. Thus, PTMA 7 nucleic acids and polypeptides, antibodies and related compounds according to the invention will be useful in therapeutic applications implicated in various cancers, coronary artery disease, arthritis, and diabetic retinopathy. In addition, oncostatin had been implicated as an inhibitor of apoptosis. Accordingly, PTMA 7 nucleic acids, polypeptides, antibodies and related compounds of the invention may be used to treat autoimmune diseases, e.g., lupus erythematosus and rheumatoid arthritis, immune deficiency disorders such as AIDS, and cancers, e.g., melanoma, cervical cancer and Burkitts lymphoma.

PTMA 8, nucleic acids and encoded polypeptides includes structural motifs that are characteristic of proteins belonging to the nerve growth factor family of proteins. Neurotrophins, such as nerve growth factor play an integral role in the growth, differentiation and maintenance of neurons. Thus, PTMA 8 nucleic acids and polypeptides, antibodies and related compounds according to the invention will be useful in therapeutic applications implicated in various neurological diseases, e.g., Parkinson's Disease, Alzheimer's, amyotrophic lateral sclerosis and

psychiatric disorders. In addition, nerve growth factor has been shown to have a role in neuroimmune interactions. Accordingly, PTMA 8 nucleic acids, polypeptides, antibodies and related compounds of the invention may be used to treat inflammatory disease, *e.g.*, keratoconjunctivitis and asthma, as well as modulate tissue remodeling.

5 Additional utilities for PTMAX nucleic acids and polypeptides according to the invention are disclosed herein.

### 1. PTMA-1

A PTMA-1 nucleic acid and polypeptide according to the invention includes the nucleic and encoded polypeptide sequence of clone AC009485\_A.

The nucleic acid sequence is 327 nucleotides in length (SEQ ID NO:1), of which nucleotides 1-327 (SEQ ID NO:1) define an open reading frame encoding a polypeptide of 109 amino acids (SEQ ID NO:2).

The AC009485\_A nucleic acid has the following sequence:

ATGTCAGATGCAGCTGTAGACACCAGCTCTGAAATCATTGCCAAGGACTTAAAGGA  
GAAGAAGGAAGTTGTGAAAGAGGCGGAAAATGGAAGAGACGCCCCTGCTAACGGG  
AATGCTAATGAGGAAAATGGGGAGCAGGAGGCTGACAAGGAGGTAGATGAAGAAG  
GGGAAGAAAGTGGGGAGGAAGAGGAGGAGGAAAAAGAAGGTGATGGTGAGGAAG  
AGGATGGAGATGAAGAGGAAGCTGAGTCTGCTACAGGCAAGCGGGCAGCTGAAGA  
TGATGAGGATGATGATGTCGATACCAAGAAGCAGAAGACCGACAAGGATGAC (SEQ  
ID NO:1)

The polypeptide encoded by clone AC009485\_A has the following sequence:

MSDAAVDTSSSEIIAKDLKEKKEVVKEAENGRDAPANGNANEENGEQEADKEVDEEGEE  
SGEEEEEEKEGDGEEEDGDEEEAESATGKRAAEDDEDDVDTKKQKTDKDD (SEQ ID  
NO:2)

The calculated molecular weight of PTMA-1 is 11909.9 daltons. Clone AC009485\_A was subjected to a search of sequence databases using BLAST programs. It was found, for



example, that the amino acid sequence of the invention has 100 of 109 residues (91%), identical to, or 103 of 109 residues (94%) positive to human prothymosin alpha having 109 amino acid residues (accession number ptrn: SPTREMBL-ACC:Q15249 PROTHYMOSIN ALPHA (PROT-ALPHA) - HOMO SAPIENS).

5 Example 2B (discussed below) shows that clone AC009485\_A is highly expressed in thymus tissue which is consistent with its identification as a thymic hormone.

## 2. PTMA-2

10 A PTMA-2 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of AC010175\_A.0.1.

The nucleic acid sequence is 555 nucleotides in length (SEQ ID NO:3), of which nucleotides 1-342 (SEQ ID NO:3) define an open reading frame encoding a polypeptide of 114 amino acids (SEQ ID NO:4).

The AC010175\_A.0.1 nucleic acid has the following sequence:

00967266-092600  
 20 ATGTCAGACGCAGCCGTAGACACCAGCTCCGAAATCACCACCGAGGACTTAAAGGA  
 GAAGAAGGAAGTTGTGGAAGAGGCGGAAAATGGAAGAGACGCCCTGCTCACGGG  
 AATGCTAATGAGGAAAATGGGGAGCCGGAGGCTGACAACGAGGTAGATGAAGAAG  
 AGGAAGAAGGTGGGGAGGAAGAAGGTGATGGTGAGGAAGAGGATGGAGATGAAGA  
 TGAGGGAGCTGAGTCAGCTACGGGCAAGCGGGCAGCTGAAGATGATGAGGATAACG  
 ATGTCGATACCCAGAAGCAGAAGACCGACGAGGATGACCAGACGGCAAAAAAGGA  
 AAAGTTAAACTAAAAAAAAGGCCGCGGTGACCTATTCACCCTCCACTTCCCGTCTC  
 AGAATCTAAACGTGGTCACCTTCGAGTAGAGGGGCCCCGCCCGCCACCGTGGGCAG  
 TGCCACCCGCAGATGACACGCGCTCTCCACCACCCAACCAAACCATGAGAATTTGC  
 25 AACAGGGGAGGGAAAAAGAACCAAACTTCCAAGGCCCTGCTTTTTTTTTTTT (SEQ  
 ID NO:3)

The polypeptide encoded by clone AC010175\_A.0.1 has the following sequence:

MSDAAVDTSSSEITTEDLKEKKEVVVEEAENGRDAPAHGNANEENGEPEADNEVDEEEEEE  
 GGGEEGDGEEEDGDEDEGAESATGKRAAEDDEDNDVDTQKQKTDEDDQTAKKEKLN  
 (SEQ ID NO:4)

The calculated molecular weight of the predicted protein is 12389.2 daltons. Clone  
 5 AC010175\_A.0.1 was subjected to a search of sequence databases using BLAST programs. It  
 was found, for example, that the amino acid sequence of the invention has 112 of 117 residues  
 (95%), identical to, or 113 of 117 residues (96%) positive to human prothymosin alpha  
 pseudogene having 117 amino acid residues (accession number ACC:AAA36485 HUMAN  
 PROTHYMOSIN-ALPHA PSEUDOGENE - HOMO SAPIENS).

### 3. PTMA-3

A PTMA-3 nucleic acid and polypeptide according to the invention includes the nucleic  
 acid and encoded polypeptide sequence of AC010175\_A.9.5.

The nucleic acid sequence is 675 nucleotides in length (SEQ ID NO:5), of which  
 nucleotides 55-397 (SEQ ID NO:5) define an open reading frame encoding a polypeptide of 114  
 amino acids (SEQ ID NO:6).

The AC010175\_A.9.5 nucleic acid has the following sequence:

TGAACTCTCGCTTTCTTTTAAATCCCCTGCATCGGATCACCGGCGTGCCCCACCATGT  
 CAGACGCAGCCGTAGACACCAGCTCCGAAATCACCAACAAGGACTTAAAGGAGAAG  
 20 AAGGAAGTTGTGGAAGAGGCAGAAAATGGAAGAGACGCCCTGCTAACGGGAATG  
 CTAATGAGGAAAATGGGGAGCAGGAGGCTGACAATGAGGTAGACGAAGAAGAGGA  
 AGAAGGTGGGGAGGAAGAAGGTGATGGTGAGGAAGAGGATGGAGATGAAGATGAG  
 GAAGCTGAGTCAGCTACGGGCAAGCGGGCAGCTGAAGATGATGAGGATAACGATGT  
 CGATACCAAGAAGCAGAAGACCGACGAGGATGACCAGACGGCAAAAAAGGAAAAG  
 25 TTAAACTAAAAAAGGCCGCGTGACCTATTCACCTCCACTTCCCGTCTCA  
 GAATCTAAACGTGGTCACCTTCGAGTAGAGAGGCCCGCCCGCCACCGTGGGCAGT  
 GCCACCCGCAGATGACACGCGCTCTCCACCACCCAACCCAAACCATGAGAATTTGC  
 AACAGGGGAGGAAAAAAGAACCAAACTTCCAAGGCCTGCTTTTTTTCTTAAAAGT

ACTTTAAAAAGGAAATTTGTTTGTATTTTTTATTTCCATTTTATATTTTTGTACATATT  
G (SEQ ID NO:5)

The polypeptide encoded by clone AC010175\_A.9.5 has the following sequence:

MSDAAVDTSSSEITNKDLKEKKEVVVEEAENGRDAPANGNANEENGEQEADNEVDEEEEE  
5 GGEEEGDGEEEDGDEDEEAESATGKRAAEDDEDNDVDTKKQKTDEDDQTAKKEKLN  
(SEQ ID NO:6)

The calculated molecular weight of the protein is 12481.4 daltons. Clone  
AC010175\_A.9.5 was subjected to a search of sequence databases using BLAST programs. It  
was found, for example, that the amino acid sequence of the invention has 106 of 117 residues  
10 (90%), identical to, or 110 of 117 residues (94%) positive to human prothymosin alpha  
pseudogene having 117 amino acid residues (accession number remtrembl-ACC:AAA36485  
HUMAN PROTHYMOSIN-ALPHA PSEUDOGENE - HOMO SAPIENS).

#### 4. PTMA-4

A PTMA-4 nucleic acid and polypeptide according to the invention includes the nucleic  
acid and encoded polypeptide sequence of AC009533\_A.

The nucleic acid sequence is 345 nucleotides in length (SEQ ID NO:7), of which  
nucleotides 1-342 (SEQ ID NO:7) define an open reading frame encoding a polypeptide of 114  
amino acids (SEQ ID NO:8).

The AC009533\_A nucleic acid has the following sequence:

atgtcagacgcagccgtagacaccagctccgaaatcaccaccgaggacttaaaggagaagaaggaagttgtggaagaggcggaaaatg  
gaagagacgcccctgctcacgggaatgctaagaggaaaatggggagccggaggctgacaacgaggtagatgaagaagaggaagaag  
gtggggaggaagaaggtgatggtgaggaagaggatggagatgaagatgagggagctgagtcagctacgggcaagcgggcagctgaa  
gatgatgaggatgacgatgtcgataccagaagcagaagaccgacgaggatgaccagacagcaaaaaaggaaaagttaaactaa  
25 (SEQ ID NO:7)

The polypeptide encoded by clone AC009533\_A has the following sequence:

MSDAAVDTSSSEITTEDLKEKKEVVVEEAENGRDAPAHGNANEENGEPEADNEVDEEEEE  
 GGGEEGDGEEEDGDEDEGAESATGKRAAEDDEDDVDVTQKQKTDEDDQTAKKEKLN  
 (SEQ ID NO:8)

The calculated molecular weight of the protein is 12390.2 daltons. Clone AC009533\_A  
 was subjected to a search of sequence databases using BLAST programs. It was found, for  
 example, that the nucleic acid sequence has 282 of 322 bases (87%) identical to human  
 prothymosin alpha gene (clone pHG4) (GENBANK-ID:HUMPROC/acc:L21695). It was found,  
 for example, that the amino acid sequence of the invention has 111 of 117 residues (94%),  
 identical to, or 113 of 117 residues (96%) positive to human prothymosin alpha pseudogene  
 (accession number ptrn:REMTREMBL-ACC:G190372); or 99 of 109 residues (90%) identical  
 to, or 102 of 109 residues (93%) positive to a sequence for human prothymosin alpha (accession  
 number ptrn:SPTREMBL-ACC:Q15249). A major distinction of the presently described protein  
 is a deletion of a run of four contiguous glutamate residues after position 63, compared to the  
 related sequences that were identified.

Example 2C (discussed below) shows that clone AC009533\_A is highly expressed in  
 thymus tissue which is consistent with its identification as a thymic hormone.

## 5. PTMA-5

A PTMA-5 nucleic acid and polypeptide according to the invention includes the nucleic  
 acid and encoded polypeptide sequence of AL121585\_A.

The nucleic acid sequence is 501 nucleotides in length (SEQ ID NO:9), of which  
 nucleotides 134-460 (SEQ ID NO:9) define an open reading frame encoding a polypeptide of  
 109 amino acids (SEQ ID NO:10). A PTMA-6 nucleotide sequence according to the invention is  
 also present in clone AL121585\_A. The sequences localize to human chromosome 20.

The AL121585\_A nucleic acid has the following sequence:

ATTGTTTCCTTGTCGGCTCCTTGCTCGCCGCAGCCGCCTTTACCGCTGCGGACTCCGG  
ACACTTCATCACCACAGTCCCTGAACTCTCGCTTTCTTTTAATCCCCTGCATCGGAT  
CACTGGTGTGCCGGACCATGTCAGACGCAGCCGTAGACACCAGCTCCGAAATCACC

ACCAAGGACTTAAAGAAGAAGGAAGCTGTGGAGGAAGCGGAAAATGGAAGAGACA  
 CCCCTGCTAATGGGAAGGCTAATGAGGAAAATGGGGAGCAGGAAGCTGACAATGAA  
 GTAGATGAAGAAGAGGAAGAAGGTGGGGAGGAAGACGAGGAGGAAGAAGAAGGC  
 GATGGTGAGGAAGAGGATGGTGATGAAGACGAGGAAGCTGAGTCCGCTACGGTCAA  
 5 GCGGGCAGCTGAAGATGATGAGAATGATGATGCCTATACCAAGAAGCAGAAGACCA  
 ACAAGGATGACTAGACAGCAAAAAAGGAAATGTTAGGAGGGTGACCTATTCA (SEQ  
 ID NO:9)

The polypeptide encoded by clone AL121585\_A has the following sequence:

MSDAAVDTSSSEITTKDLKKKEAVEEAENGRDTPANGKANEENGEQADNEVDEEEEEEG  
 10 GEEDEEEEEEGDGEEEDGDEDEEAESATVKRAAEDDENDDAYTKKQKTNKDD (SEQ ID  
 NO:10)

The calculated molecular weight of the protein is 12005.8 daltons. Clone AL121585\_A  
 was subjected to a search of sequence databases using BLAST programs. It was found, for  
 example, that the nucleotide sequence of the invention has 496 of 501 bases (99%) identical to,  
 or 496 of 501 bases (99%) positive to human prothymosin alpha pseudogene (accession number  
 gb:GENBANK-ID:HUMPROAD/acc:J04800 HUMAN PROTHYMOSIN-ALPHA  
 PSEUDOGENE - HOMO SAPIENS). It was found, for example, that the amino acid sequence  
 of the invention has 99 of 110 residues (90%) identical to, or 103 of 110 residues (93%) positive  
 to human prothymosin alpha (accession number ptnr:PIR-ID:TNHUA PROTHYMOSIN-  
 20 ALPHA - HUMAN).

## 6. PTMA-6

A PTMA-6 nucleic acid and polypeptide according to the invention includes the nucleic  
 acid and encoded polypeptide sequence of clone AC010175.

25 The nucleic acid sequence is 342 nucleotides in length (SEQ ID NO:11), of which  
 nucleotides 1-342 (SEQ ID NO:11) define an open reading frame encoding a polypeptide of 114  
 amino acids (SEQ ID NO:12).

The AC010175 nucleic acid and encoded polypeptide have the following sequences:

1 ATGTCAGACGCAGCCGTAGACACCAGCTCCGAAATCACCACCGAG

MetSerAspAlaAlaValAspThrSerSerGluIleThrThrGlu

46 GACTTAAAGGAGAAGAAGGAAGTTGTGGAAGAGGCGGAAAATGGA

AspLeuLysGluLysLysGluValValGluGluAlaGluAsnGly

5 91 AGAGACGCCCCTGCTCACGGGAATGCTAATGAGGAAAATGGGGAG

ArgAspAlaProAlaHisGlyAsnAlaAsnGluGluAsnGlyGlu

136 CCGGAGGCTGACAACGAGGTAGATGAAGAAGAGGAAGAAGGTGGG

ProGluAlaAspAsnGluValAspGluGluGluGluGlyGly

181 GAGGAAGAAGGTGATGGTGAGGAAGAGGATGGAGATGAAGATGAG

GluGluGluGlyAspGlyGluGluGluAspGlyAspGluAspGlu

226 GGAGCTGAGTCAGCTACGGGCAAGCGGGCAGCTGAAGATGATGAG

GlyAlaGluSerAlaThrGlyLysArgAlaAlaGluAspAspGlu

271 GATAACGATGTCGATACCCAGAAGCAGAAGACCGACGAGGATGAC

AspAsnAspValAspThrGlnLysGlnLysThrAspGluAspAsp

316 CAGACGGCAAAAAAGGAAAAGTTAAAC (SEQ ID NO:11)

GlnThrAlaLysLysGluLysLeuAsn (SEQ ID NO:12)

The calculated molecular weight of the protein is 12389.2 daltons. Clone AC010175 was subjected to a search of sequence databases using BLAST programs. It was found, for example, that the amino acid sequence of the invention has 98 of 109 residues (89%) identical to, or 102 of 109 residues (93%) positive to human prothymosin alpha a sequence for human prothymosin alpha which is disclosed, for example, in US Patents 4,659,694 and 4,716,148.

Example 2A (discussed below) shows that clone AC010175 is highly expressed in thymus tissue which is consistent with its identification as a thymic hormone.

## 7. PTMA-7

A PTMA-7 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone AC010784-1.

The nucleic acid sequence is 324 nucleotides in length (SEQ ID NO:13), of which nucleotides 1-324 (SEQ ID NO:13) define an open reading frame encoding a polypeptide of 108 amino acids (SEQ ID NO:14).

The AC010784-1 nucleic acid and encoded polypeptide have the following sequences:

1 ATGAGCTCCGCCAGCCGGGTTTTGCGCCTTCAGGCCCCCGGGTTG

MetSerSerAlaSerArgValLeuArgLeuGlnAlaProGlyLeu

46 GTGTTCTGTTGGGTTGGTGCTCCTTTCCCTCCCCTCGTCCTCTCTT

ValPheLeuGlyLeuValLeuLeuSerLeuProSerSerSerLeu

91 ACCCTCTCCATTTCCCCCTCAGCTGAAGCTGAAGAAGATGGGGAC

ThrLeuSerIleSerProSerAlaGluAlaGluGluAspGlyAsp

136 CTGCAGTGCCTGTGTGTGAAGACCACCTCCCAGGTCCGTCCCAGG

LeuGlnCysLeuCysValLysThrThrSerGlnValArgProArg

181 CACATCACCAGCCTGGAGGTGATCAAGGCCGGACCCCACTGCCCC

HisIleThrSerLeuGluValIleLysAlaGlyProHisCysPro

226 ACTGCCCAACTGATGGCCACGCTGAAGAATGGAAGGAAAATTTGC

ThrAlaGlnLeuMetAlaThrLeuLysAsnGlyArgLysIleCys

271 TTGGACCTGCAAGCCCCGCTGTACAAGAAAAGGATTAAGAAACTT

LeuAspLeuGlnAlaProLeuTyrLysLysArgIleLysLysLeu

316 TTGAAGAGT (SEQ ID NO:13)

LeuLysSer (SEQ ID NO:14)

The calculated molecular weight of the protein is 11680.7 daltons. Clone AC010784-1 was subjected to a search of sequence databases using BLAST programs. It was found, for example, that the amino acid sequence of the invention has 84 of 108 residues (77%) identical to,

or 93 of 108 residues (86%) positive to a sequence for platelet factor 4 (PF-4) (oncostatin A). Such related nucleic acids and proteins are disclosed, for example, by Poncz, M., Surrey, S., LaRocco, P., Weiss, M. J., Rappaport, E. F., Conway, T. M. and Schwartz, E. (Blood 69 (1), 219-223 (1987)), and in US Patent 5,656,724.

The novel oncostatin A-like polypeptide of the present invention may serve as a novel growth-modulating factor to which various cells and tissues in the human body respond. The invention is therefore useful in potential therapeutic applications, for a cDNA encoding the oncostatin A-like polypeptide may be useful in gene therapy, and the oncostatin A-like polypeptide may be useful when administered to a subject in need thereof. The novel nucleic acid encoding oncostatin A-like polypeptide, and the polypeptide of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the polypeptide are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention in therapeutic or diagnostic methods.

#### 8. PTMA-8

A PTMA-8 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone AL049825.

The nucleic acid sequence is 738 nucleotides in length (SEQ ID NO:15), of which nucleotides 13 to 735 (SEQ ID NO:15) define an open reading frame encoding a polypeptide of 241 amino acids (SEQ ID NO:16).

The AL049825 nucleic acid and encoded polypeptide has the following sequence:

1 GTGCATAGCGTAATGTCCATGTTGTTCTACACTCTGATCACAGCT

MetSerMetLeuPheTyrThrLeuIleThrAla

46 TTTCTGATCGGCATACAGGCGGAACCACACTCAGAGAGCAATGTC

PheLeuIleGlyIleGlnAlaGluProHisSerGluSerAsnVal

91 CCTGCAGGACACACCATCCCCCAAGCCCACTGGACTAAACTTCAG



ProAlaGlyHisThrIleProGlnAlaHisTrpThrLysLeuGln

136 CATTCCCTTGACACTGCCCTTCGCAGAGCCCGCAGCGCCCCGGCA

HisSerLeuAspThrAlaLeuArgArgAlaArgSerAlaProAla

181 GCGGCGATAGCTGCACGCGTGGCGGGGCAGACCCGCAACATTACT

5 AlaAlaIleAlaAlaArgValAlaGlyGlnThrArgAsnIleThr

226 GTGGACCCCAGGCTGTTTAAAAAGCGGCGACTCCGTTCACCCCGT

ValAspProArgLeuPheLysLysArgArgLeuArgSerProArg

271 GTGCTGTTTAGCACCCAGCCTCCCCGTGAAGCTGCAGACACTCAG

ValLeuPheSerThrGlnProProArgGluAlaAlaAspThrGln

316 GATCTGGACTTCGAGGTCGGTGGTGCTGCCCCCTTCAACAGGACT

AspLeuAspPheGluValGlyGlyAlaAlaProPheAsnArgThr

361 CACAGGAGCAAGCGGTCATCATCCCATCCCATCTTCCACAGGGGC

HisArgSerLysArgSerSerSerHisProIlePheHisArgGly

406 GAATTCTCGGTGTGTGACAGTGTGACGCGTGTGGGTGTTGGGGATAAG

GluPheSerValCysAspSerValSerValTrpValGlyAspLys

451 ACCACCGCCACAGACATCAAGGGCAAGGAGGTGATGGTGTGTTGGA

ThrThrAlaThrAspIleLysGlyLysGluValMetValLeuGly

496 GAGGTGAACATTAACAACAGTGTATTCAAACAGTACTTTTTTGAG

GluValAsnIleAsnAsnSerValPheLysGlnTyrPhePheGlu

20 541 ACCAAGTGCCGGGACCCAAATCCCGTTGACAGCGGGTGCCGGGGC

ThrLysCysArgAspProAsnProValAspSerGlyCysArgGly

586 ATTGACTCAAAGCACTGGAACATCATATTGTACCACGACTCACACC

IleAspSerLysHisTrpAsnSerTyrCysThrThrThrHisThr

631 TTTGTCAAGGCGCTGACCATGGATGGCAAGCAGGCTGCCTGGCGG

PheValLysAlaLeuThrMetAspGlyLysGlnAlaAlaTrpArg

676 TTTATCCGGATAGATACGGCCTGTGTGTGTGTGCTCAGCAGGAAG

PhelleArgIleAspThrAlaCysValCysValLeuSerArgLys

5 721 GCTGTGAGAAGAGCCTGA (SEQ ID NO:15)

AlaValArgArgAla (SEQ ID NO:16)

The calculated molecular weight of the protein is 26958.5 daltons. Clone AL049825 was subjected to a search of sequence databases using BLAST programs. It was found, for example, that the amino acid sequence of the invention has 240 of 241 residues (99.5%) similar to a 241 residue sequence for human beta-nerve growth factor precursor (SWISSPROT-ACC:P01138).

This human beta-nerve growth factor precursor-like nucleic acid and polypeptide is also similar to a nucleic acid and polypeptide in PCT publication WO9821234. The protein of this invention includes an alanine at position 35, which differs from the disclosed protein in that a valine appears at this position. According to WO9821234, the prepro region of the polypeptide extends from residue 1 to 121. Thus the variant of the present invention may be implicated in pathological conditions which could arise from inappropriate or incorrect processing. Were this to occur, either the secretion of the protein from one intracellular compartment to another or to the external medium, or the folding of the mature domain of the nerve growth factor beta chain could be adversely affected. Therefore nucleotide sequences and peptide or protein sequences characteristic of the variant of the present invention would find use in diagnostic screening methods, as well as in methods of treating neurological disorders, and in screening for therapeutics that would overcome any pathological state associated with the occurrence of the variant gene and/or its gene product.

## 9. PTMA-9

APTMA-9 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone AL121585\_da1.

The nucleic acid sequence is 345 nucleotides in length (SEQ ID NO:17), of which nucleotides 10-339 (SEQ ID NO:17) define an open reading frame encoding a polypeptide of 110 amino acids (SEQ ID NO:18).

The AL121585\_da1 nucleic acid has the following sequence:

5 TGCCGGACCATGTCAGACGCAGCCGTAGACACCAGCTCCGAAATCACCACCAAGGA  
CTTAAAGGAGAAGAAGGAAGTTGTGGAAGAGGCAGAAAATGGAAGAGACGCCCT  
GCTAACGGGAATGCTAATGAGGAAAATGGGGAGCAGGAGGCTGACAATGAGGTAG  
ACGAAGAAGAGGAAGAAGGTGGGGAGGAAGAGGAGGAGGAAGAAGAAGGTGATG  
GTGAGGAAGAGGATGGAGATGAAGATGAGGAAGCTGAGTCAGCTACGGGCAAGCG  
10 GGCAGCTGAAGATGATGAGGATGACGATGTCGATACCAAGAAGCAGAAGACCAAC  
AAGGATGACTAGACA (SEQ ID NO:17).

The AL121585\_da1 polypeptide has the following sequence (using the one-letter amino acid code):

MSDAAVDTSSEITTKDLKEKKEVVEEAENGRDAPANGNANEENGEQEADNEVD  
EEEEEGGEEEEEEEEEGDGEEEDGDEDEEAESATGKRAAEDDEDDVDTKKQKTNKDD  
(SEQ ID NO:18).

The calculated molecular weight of the protein is 12071.8 daltons. Clone AC010175 was subjected to a search of sequence databases using BLAST programs. It was found, for example, that the amino acid sequence of the invention has 108 of 110 residues (98%) identical to, or all 110 residues (100%) positive to human prothymosin alpha (PIR ID:TNHUA).

## 10. PTMA-10

A PTMA-10 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone AL121585\_da2.

25 The nucleic acid sequence is 350 nucleotides in length (SEQ ID NO:19), of which nucleotides 10-348 (SEQ ID NO:19) define an open reading frame encoding a polypeptide of 113 amino acids (SEQ ID NO:20).

The AL121585\_da2 nucleic acid has the following sequence:

TGCCGGACCATGTCAGACGCAGCCGTACACACCACCTCCGAAATCACCACCAAGGA  
 CTTAAAGGAGAAGAAGGAAGTTGTGGAAGAGGCAGAAAATGGAAGAGACGCCCCCT  
 GCTAACGGGAATGCTAATGAGGAAAATGGGGAGCAGGAGGCTGACAATGAGGTAG  
 5 ACCAAGAAGAGGAAGAAGGTGGGGAGGAAGAGGAGGAGGAAGAAGAAGGTGATG  
 GTGAGGAAGAGGATGGAGATGAAGATGAGGAAGCTGAGTCACCTACGGGCAACCG  
 GGCAGCTGAAGATGATGAGGATGACGATGTCAATACCAAGGAAGGCGGAAGGACC  
 AACCAAGGGATGACTAGACA (SEQ ID NO:19).

The AL121585\_da2 polypeptide has the following sequence (using the one-letter amino  
 10 acid code):

MSDAAVHTTSEITTKDLKEKKEVVEEAENGRDAPANGNANEENGEQEADNEVDQEEEE  
 GGEEEEEEEEEGDGEEEDGDEDEEAESPTGNRAAEDDEDDVDVNTKEGGRTNQGMTR  
 (SEQ ID NO:20)

The calculated molecular weight of the protein is 12348.2 daltons. Clone AL121585\_da2  
 was subjected to a search of sequence databases using BLAST programs. It was found, for  
 example, that the amino acid sequence of the invention has 96 of 103 residues (93%) identical to,  
 or 100 of 1039 residues (97%) positive to a 110 residue human prothymosin alpha (PIR  
 ID:TNHUA).

## PTMAX Nucleic Acids

One aspect of the invention pertains to isolated nucleic acid molecules that encode  
 PTMAX polypeptides or biologically active portions thereof. Also included in the invention are  
 nucleic acid fragments sufficient for use as hybridization probes to identify PTMAX-encoding  
 25 nucleic acids (*e.g.*, PTMAX mRNA) and fragments for use as PCR primers for the amplification  
 or mutation of PTMAX nucleic acid molecules. As used herein, the term "nucleic acid molecule"  
 is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*,

mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

“Probes” refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An “isolated” nucleic acid molecule is one that is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an “isolated” nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated PTMAX nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 as a hybridization probe, PTMAX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2<sup>nd</sup> Ed., Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR  
5 amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to PTMAX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues,  
10 which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to  
30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or a portion of this nucleotide sequence, *e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically active portion of PTMAX. A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 is one that is sufficiently complementary to the nucleotide  
25 sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotide units of a nucleic acid molecule, and the term "binding" means the

physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound.

- 5 Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 30%, 50%, 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

5 A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of PTMAX polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a PTMAX polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, *e.g.*, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human PTMAX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 as well as a polypeptide having PTMAX activity. Biological activities of the PTMAX proteins are described below. A homologous amino acid sequence does not encode the amino acid sequence of a human PTMAX polypeptide.

10 A PTMAX polypeptide is encoded by the open reading frame ("ORF") of a PTMAX nucleic acid. The invention includes the nucleic acid sequence comprising the stretch of nucleic acid sequences of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 that comprises the ORF of that nucleic acid sequence and encodes a polypeptide of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

25 An "open reading frame" ("ORF") corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a bona fide cellular protein, a minimum size requirement is often set, for example, a stretch of DNA that would encode a protein of 50 amino acids or more.



The nucleotide sequence determined from the cloning of the human PTMAX gene allows for the generation of probes and primers designed for use in identifying and/or cloning PTMAX homologues in other cell types, *e.g.* from other tissues, as well as PTMAX homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or an anti-sense strand nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or of a naturally occurring mutant of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19.

Probes based on the human PTMAX nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a PTMAX protein, such as by measuring a level of a PTMAX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting PTMAX mRNA levels or determining whether a genomic PTMAX gene has been mutated or deleted.

"A polypeptide having a biologically active portion of PTMAX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of PTMAX" can be prepared by isolating a portion of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 that encodes a polypeptide having a PTMAX biological activity (the biological activities of the PTMAX proteins are described below), expressing the encoded portion of PTMAX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of PTMAX.

### **PTMAX variants**

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 due to degeneracy of the

genetic code and thus encode the same PTMAX protein as that encoded by the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

5 In addition to the human PTMAX nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of PTMAX may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the PTMAX gene may exist among individuals within a population due to natural allelic variation. As used herein, the  
10 terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an PTMAX protein, preferably a mammalian PTMAX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the PTMAX gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in PTMAX that are the result of natural allelic variation and that do not alter the functional activity of PTMAX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding PTMAX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the PTMAX cDNAs of the invention can be isolated based on their homology to the human PTMAX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human PTMAX cDNA can be isolated based on its homology to human membrane-bound PTMAX. Likewise, a membrane-bound human PTMAX cDNA can be isolated  
25 based on its homology to soluble human PTMAX.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or

2000 or more nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding PTMAX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at  $T_m$ , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high

salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792.

## Conservative mutations

In addition to naturally-occurring allelic variants of a PTMAX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, thereby leading to changes in the amino acid sequence of the encoded PTMAX protein, without altering the functional ability of the PTMAX protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of PTMAX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the PTMAX proteins of the present invention, are predicted to be particularly unamenable to alteration. Amino acids for which conservative substitutions can be made are known in the art.

Another aspect of the invention pertains to nucleic acid molecules encoding PTMAX proteins that contain changes in amino acid residues that are not essential for activity. Such PTMAX proteins differ in amino acid sequence from SEQ IDs NO:2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 20. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, more preferably at least about 70% homologous to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, still more preferably at least about 80% homologous to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, even more preferably at least about 90% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 and most preferably at least about 95% homologous to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

An isolated nucleic acid molecule encoding an PTMAX protein homologous to the protein of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:

2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ IDs NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

5 Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (10 *e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in PTMAX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an PTMAX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for PTMAX biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant PTMAX protein can be assayed for (1) the ability to form protein:protein interactions with other PTMAX proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant PTMAX protein and an PTMAX ligand; (3) the ability of a mutant PTMAX protein to bind to an intracellular (25 target protein or biologically active portion thereof; (*e.g.* avidin proteins).

### Antisense

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide

sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire PTMAX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an PTMAX protein of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, or antisense nucleic acids complementary to an PTMAX nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding PTMAX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding PTMAX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding PTMAX disclosed herein antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of PTMAX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of PTMAX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of PTMAX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability

of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an PTMAX protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic



acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

5 In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

### Ribozymes and PNA moieties

Nucleic acid modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

10 In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave PTMAX mRNA transcripts to thereby inhibit translation of PTMAX mRNA. A ribozyme having specificity for an PTMAX-encoding nucleic acid can be designed based upon the nucleotide sequence of an PTMAX cDNA disclosed  
25 herein (*i.e.*, SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an PTMAX-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742.

Alternatively, PTMAX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, PTMAX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the PTMAX (*e.g.*, the PTMAX promoter and/or enhancers) to form triple helical structures that prevent transcription of the PTMAX gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of PTMAX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of PTMAX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of PTMAX can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of PTMAX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug

delivery known in the art. For example, PNA-DNA chimeras of PTMAX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

### PTMAX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of PTMAX polypeptides whose sequences are provided by SEQ IDs NO:2, 4, 6, 8, 10, 12, 14, 16, 18, and 20. The invention also includes a mutant or variant protein any of whose

residues may be changed from the corresponding residues shown in SEQ IDs NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 while still encoding a protein that maintains its PTMAX activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

5 In general, a PTMAX variant that preserves PTMAX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

0082260-092800 09672685-092800 One aspect of the invention pertains to isolated PTMAX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-PTMAX antibodies. In one embodiment, native PTMAX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, PTMAX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a PTMAX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

20 An "isolated" or "purified" polypeptide or protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PTMAX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PTMAX protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one  
25 embodiment, the language "substantially free of cellular material" includes preparations of PTMAX protein having less than about 30% (by dry weight) of non-PTMAX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-PTMAX protein, still more preferably less than about 10% of non-PTMAX protein, and

most preferably less than about 5% non-PTMAX protein. When the PTMAX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

5           The language "substantially free of chemical precursors or other chemicals" includes preparations of PTMAX protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of PTMAX protein having less than about 30% (by dry weight) of chemical precursors or  
10 non-PTMAX chemicals, more preferably less than about 20% chemical precursors or non-PTMAX chemicals, still more preferably less than about 10% chemical precursors or non-PTMAX chemicals, and most preferably less than about 5% chemical precursors or non-PTMAX chemicals.

Biologically active portions of a PTMAX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the PTMAX protein, *e.g.*, the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, that include fewer amino acids than the full length PTMAX proteins, and exhibit at least one activity of an PTMAX protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the PTMAX protein. A biologically active portion of a  
20 PTMAX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native PTMAX protein.

25           In an embodiment, the PTMAX protein has an amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20. In other embodiments, the PTMAX protein is substantially homologous to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 and retains the functional activity of the protein of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below.

Accordingly, in another embodiment, the PTMAX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 and retains the functional activity of the PTMAX proteins of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

## 5 Determining homology between two or more sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, Needleman and Wunsch 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window

size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

### Chimeric and fusion proteins

The invention also provides PTMAX chimeric or fusion proteins. As used herein, an PTMAX "chimeric protein" or "fusion protein" comprises a PTMAX polypeptide operatively linked to a non-PTMAX polypeptide. A "PTMAX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to PTMAX, whereas a "non-PTMAX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the PTMAX protein, *e.g.*, a protein that is different from the PTMAX protein and that is derived from the same or a different organism. Within a PTMAX fusion protein the PTMAX polypeptide can correspond to all or a portion of a PTMAX protein. In one embodiment, a PTMAX fusion protein comprises at least one biologically active portion of a PTMAX protein. In another embodiment, a PTMAX fusion protein comprises at least two biologically active portions of a PTMAX protein. In yet another embodiment, a PTMAX fusion protein comprises at least three biologically active portions of a PTMAX protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the PTMAX polypeptide and the non-PTMAX polypeptide are fused in-frame to each other. The non-PTMAX polypeptide can be fused to the N-terminus or C-terminus of the PTMAX polypeptide.

In one embodiment, the fusion protein is a GST-PTMAX fusion protein in which the PTMAX sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant PTMAX.

In another embodiment, the fusion protein is a PTMAX protein containing a heterologous signal sequence at its N-terminus. For example, the native PTMA-7 signal sequence (*i.e.*, about amino acids 1 to 40 of SEQ ID NO:14) can be removed and replaced with a signal sequence from

another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of PTMAX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is a PTMAX-immunoglobulin fusion protein in which the PTMAX sequences are fused to sequences derived from a member of the immunoglobulin protein family. The PTMAX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a PTMAX ligand and an PTMAX protein on the surface of a cell, to thereby suppress PTMAX-mediated signal transduction *in vivo*. The PTMAX-immunoglobulin fusion proteins can be used to affect the bioavailability of a PTMAX cognate ligand. Inhibition of the PTMAX ligand/PTMAX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the PTMAX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-PTMAX antibodies in a subject, to purify PTMAX ligands, and in screening assays to identify molecules that inhibit the interaction of PTMAX with an PTMAX ligand.

A PTMAX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A PTMAX-encoding nucleic acid can



be cloned into such an expression vector such that the fusion moiety is linked in-frame to the PTMAX protein.

### PTMAX agonists and antagonists

The present invention also pertains to variants of the PTMAX proteins that function as either PTMAX agonists (mimetics) or as PTMAX antagonists. Variants of the PTMAX protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the PTMAX protein. An agonist of the PTMAX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the PTMAX protein. An antagonist of the PTMAX protein can inhibit one or more of the activities of the naturally occurring form of the PTMAX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the PTMAX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the PTMAX proteins.

Variants of the PTMAX protein that function as either PTMAX agonists (mimetics) or as PTMAX antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the PTMAX protein for PTMAX protein agonist or antagonist activity. In one embodiment, a variegated library of PTMAX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of PTMAX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential PTMAX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of PTMAX sequences therein. There are a variety of methods which can be used to produce libraries of potential PTMAX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential PTMAX sequences.

Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

### Polypeptide libraries

5 In addition, libraries of fragments of the PTMAX protein coding sequence can be used to generate a variegated population of PTMAX fragments for screening and subsequent selection of variants of a PTMAX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an PTMAX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the  
10 double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the PTMAX protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of PTMAX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene  
20 libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can  
25 be used in combination with the screening assays to identify PTMAX variants (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6:327-331).

## Anti-PTMAX Antibodies

The invention encompasses antibodies and antibody fragments, such as  $F_{ab}$  or  $(F_{ab})_2$ , that bind immunospecifically to any of the polypeptides of the invention.

An isolated PTMAX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind PTMAX using standard techniques for polyclonal and monoclonal antibody preparation. The full-length PTMAX protein can be used or, alternatively, the invention provides antigenic peptide fragments of PTMAX for use as immunogens. The antigenic peptide of PTMAX comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 and encompasses an epitope of PTMAX such that an antibody raised against the peptide forms a specific immune complex with PTMAX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of PTMAX that is located on the surface of the protein, *e.g.*, a hydrophilic region. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety.

As disclosed herein, PTMAX protein sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as PTMAX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain,  $F_{ab}$  and  $F_{(ab)2}$  fragments, and an  $F_{ab}$  expression library. In a specific embodiment, antibodies to

human PTMAX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a PTMAX protein sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20, or a derivative, fragment, analog or homolog thereof.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed PTMAX protein or a chemically synthesized PTMAX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against PTMAX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of PTMAX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular PTMAX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular PTMAX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by

transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a PTMAX protein (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F<sub>ab</sub> expression libraries (see *e.g.*, Huse, *et al.*, 1989 *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F<sub>ab</sub> fragments with the desired specificity for a PTMAX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See *e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to a PTMAX protein may be produced by techniques known in the art including, but not limited to: (i) an F<sub>(ab)<sub>2</sub></sub> fragment produced by pepsin digestion of an antibody molecule; (ii) an F<sub>ab</sub> fragment generated by reducing the disulfide bridges of an F<sub>(ab)<sub>2</sub></sub> fragment; (iii) an F<sub>ab</sub> fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F<sub>v</sub> fragments.

Additionally, recombinant anti-PTMAX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J Natl Cancer Inst* 80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science*

239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a PTMAX protein is facilitated by generation of hybridomas that bind to the fragment of a PTMAX protein possessing such a domain. Thus, antibodies that are specific for a desired domain within a PTMAX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-PTMAX antibodies may be used in methods known within the art relating to the localization and/or quantitation of an PTMAX protein (*e.g.*, for use in measuring levels of the PTMAX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for PTMAX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-PTMAX antibody (*e.g.*, monoclonal antibody) can be used to isolate PTMAX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-PTMAX antibody can facilitate the purification of natural PTMAX from cells and of recombinantly produced PTMAX expressed in host cells. Moreover, an anti-PTMAX antibody can be used to detect PTMAX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the PTMAX protein. Anti-PTMAX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein,

fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

## 5 PTMAX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding PTMAX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that

allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in

5 Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on

10 such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., PTMAX proteins, mutant forms of PTMAX, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of PTMAX in prokaryotic or eukaryotic cells. For example, PTMAX can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve

25 three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion



protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E  
5 binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

10 One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the PTMAX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, PTMAX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*,  
25 SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include

pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to PTMAX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of

antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, PTMAX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that

encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding PTMAX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) PTMAX protein. Accordingly, the invention further provides methods for producing PTMAX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding PTMAX has been introduced) in a suitable medium such that PTMAX protein is produced. In another embodiment, the method further comprises isolating PTMAX from the medium or the host cell.

### Transgenic animals

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which PTMAX-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous PTMAX sequences have been introduced into their genome or homologous recombinant animals in which endogenous PTMAX sequences have been altered. Such animals are useful for studying the function and/or activity of PTMAX and for identifying and/or evaluating modulators of PTMAX activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous

recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous PTMAX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

5           A transgenic animal of the invention can be created by introducing PTMAX-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human PTMAX cDNA sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 or 19 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman  
10           homologue of the human PTMAX gene, such as a mouse PTMAX gene, can be isolated based on hybridization to the human PTMAX cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the PTMAX transgene to direct expression of PTMAX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder  
20           animal can be identified based upon the presence of the PTMAX transgene in its genome and/or expression of PTMAX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding PTMAX can further be bred to other transgenic animals carrying other transgenes.

25           To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a PTMAX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the PTMAX gene. The PTMAX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19), but more preferably, is a non-human homologue of a human PTMAX gene. For example, a mouse homologue of human

PTMAX gene of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19 can be used to construct a homologous recombination vector suitable for altering an endogenous PTMAX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous PTMAX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous PTMAX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous PTMAX protein). In the homologous recombination vector, the altered portion of the PTMAX gene is flanked at its 5' and 3' ends by additional nucleic acid of the PTMAX gene to allow for homologous recombination to occur between the exogenous PTMAX gene carried by the vector and an endogenous PTMAX gene in an embryonic stem cell. The additional flanking PTMAX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) *Cell* 51:503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced PTMAX gene has homologously recombined with the endogenous PTMAX gene are selected (see *e.g.*, Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

5 In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, *e.g.*, Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

10 Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

### 20 **Pharmaceutical Compositions**

25 The PTMAX nucleic acid molecules, PTMAX proteins, and anti-PTMAX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is

incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can



be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an PTMAX protein or anti-PTMAX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as

sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives.

Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired

therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

### Uses and Methods of the Invention

The isolated nucleic acid molecules of the invention can be used to express PTMAX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect PTMAX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an PTMAX gene, and to modulate PTMAX activity, as described further below. In addition, the PTMAX proteins can be used to screen drugs or compounds that modulate the PTMAX activity or expression as well as to treat disorders characterized by insufficient or excessive production of PTMAX protein or production of PTMAX protein forms that have decreased or aberrant activity compared to PTMAX wild type protein (*e.g.* proliferative disorders such as cancer and immune disorders, *e.g.*, multiple sclerosis). In addition, the anti-PTMAX antibodies of the invention can be used to detect and isolate PTMAX proteins and modulate PTMAX activity.

This invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described herein.

### Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to PTMAX proteins or have a stimulatory or inhibitory effect on, for example, PTMAX expression or PTMAX activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of a PTMAX protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon containing) or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* (1994) *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* (1994) *J Med Chem* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2059; Carell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* (1994) *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), on chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner above.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of PTMAX protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a PTMAX protein is determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the PTMAX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the PTMAX protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of PTMAX protein, or a biologically active portion thereof, on the cell surface with a known compound which binds PTMAX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PTMAX protein, wherein determining the ability of the test compound to interact with a PTMAX protein comprises determining the ability of the test compound to preferentially bind to PTMAX or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of PTMAX protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to

modulate (*e.g.*, stimulate or inhibit) the activity of the PTMAX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of PTMAX or a biologically active portion thereof can be accomplished, for example, by determining the ability of the PTMAX protein to bind to or interact with an PTMAX target molecule. As used herein, a "target molecule" is a molecule with which an PTMAX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an PTMAX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane, a molecule associated with the nuclear membrane, a molecule in the nucleus, or a cytoplasmic molecule. A PTMAX target molecule can be a non-PTMAX molecule or a PTMAX protein or polypeptide of the present invention.

In one embodiment, a PTMAX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound PTMAX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with PTMAX.

Determining the ability of the PTMAX protein to bind to or interact with a PTMAX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the PTMAX protein to bind to or interact with a PTMAX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a PTMAX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cell death, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a PTMAX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the PTMAX protein or

biologically active portion thereof. Binding of the test compound to the PTMAX protein can be determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the PTMAX protein or biologically active portion thereof with a known compound which binds PTMAX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PTMAX protein, wherein determining the ability of the test compound to interact with a PTMAX protein comprises determining the ability of the test compound to preferentially bind to PTMAX or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting PTMAX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the PTMAX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of PTMAX can be accomplished, for example, by determining the ability of the PTMAX protein to bind to a PTMAX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of PTMAX can be accomplished by determining the ability of the PTMAX protein to further modulate a PTMAX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the PTMAX protein or biologically active portion thereof with a known compound which binds PTMAX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PTMAX protein, wherein determining the ability of the test compound to interact with a PTMAX protein comprises determining the ability of the PTMAX protein to preferentially bind to or modulate the activity of a PTMAX target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-bound form of PTMAX. In the case of cell-free assays comprising the membrane-bound form of PTMAX, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of PTMAX is maintained in solution. Examples of such solubilizing

agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either PTMAX or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to PTMAX, or interaction of PTMAX with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-PTMAX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or PTMAX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of PTMAX binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either PTMAX or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated PTMAX or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with



PTMAX or target molecules, but which do not interfere with binding of the PTMAX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or PTMAX trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of  
 5 complexes using antibodies reactive with the PTMAX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the PTMAX or target molecule.

In another embodiment, modulators of PTMAX expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PTMAX mRNA or protein in the cell is determined. The level of expression of PTMAX mRNA or protein in the  
 10 presence of the candidate compound is compared to the level of expression of PTMAX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PTMAX expression based on this comparison. For example, when expression of PTMAX mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PTMAX mRNA or protein expression. Alternatively, when expression of PTMAX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PTMAX mRNA or protein expression. The level of PTMAX mRNA or protein expression in the cells can  
 20 be determined by methods described herein for detecting PTMAX mRNA or protein.

In yet another aspect of the invention, the PTMAX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J Biol Chem* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent  
 25 WO94/10300), to identify other proteins that bind to or interact with PTMAX ("PTMAX-binding proteins" or "PTMAX-bp") and modulate PTMAX activity. Such PTMAX-binding proteins are also likely to be involved in the propagation of signals by the PTMAX proteins as, for example, upstream or downstream elements of the PTMAX pathway.

09672955 - 092800

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for PTMAX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a PTMAX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with PTMAX.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

### Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the PTMAX, sequences, described herein, can be used to map the location of the PTMAX genes, respectively, on a chromosome. The mapping of the PTMAX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, PTMAX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the PTMAX sequences. Computer analysis of the PTMAX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those

hybrids containing the human gene corresponding to the PTMAX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the PTMAX sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the PTMAX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

### Tissue Typing

The PTMAX sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Pat. No. 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the PTMAX sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The PTMAX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, 3, 5 or 7 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:9, 10, 11, or 12 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

### **Predictive Medicine**

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining PTMAX protein and/or nucleic acid expression as well as PTMAX activity, in the context of a biological sample

(*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant PTMAX expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with PTMAX protein, nucleic acid expression or activity. For example, mutations in a PTMAX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with PTMAX protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining PTMAX protein, nucleic acid expression or PTMAX activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of PTMAX in clinical trials.

These and other agents are described in further detail in the following sections.

### Diagnostic Assays

An exemplary method for detecting the presence or absence of PTMAX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting PTMAX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes PTMAX protein such that the presence of PTMAX is detected in the biological sample. An agent for detecting PTMAX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to PTMAX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length PTMAX nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to

specifically hybridize under stringent conditions to PTMAX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

5 An agent for detecting PTMAX protein is an antibody capable of binding to PTMAX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a  
10 primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect PTMAX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of PTMAX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of PTMAX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of PTMAX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of PTMAX protein include introducing into a subject a labeled anti-PTMAX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

15 In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting PTMAX protein, mRNA, or genomic DNA, such that the presence of PTMAX

protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of PTMAX protein, mRNA or genomic DNA in the control sample with the presence of PTMAX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of PTMAX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting PTMAX protein or mRNA in a biological sample; means for determining the amount of PTMAX in the sample; and means for comparing the amount of PTMAX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect PTMAX protein or nucleic acid.

## 10 Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant PTMAX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with PTMAX protein, nucleic acid expression or activity such as cancer, immune system associated (*e.g.*, multiple sclerosis), or fibrotic disorders.. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant PTMAX expression or activity in which a test sample is obtained from a subject and PTMAX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of PTMAX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant PTMAX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant PTMAX expression or activity. For example, such methods can be used



to determine whether a subject can be effectively treated with an agent for a disorder, such as cancer, immune system associated disorders, *e.g.*, multiple sclerosis. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant PTMAX expression or activity in which a test sample is

5 obtained and PTMAX protein or nucleic acid is detected (*e.g.*, wherein the presence of PTMAX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant PTMAX expression or activity.)

The methods of the invention can also be used to detect genetic lesions in an PTMAX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder

10 characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an PTMAX-protein, or the mis-expression of the PTMAX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from an PTMAX gene; (2) an addition of one or more nucleotides to an PTMAX gene; (3) a substitution of one or more nucleotides of an PTMAX gene, (4) a chromosomal rearrangement of an PTMAX gene; (5) an alteration in the level of a messenger RNA transcript of an PTMAX gene, (6) aberrant modification of an PTMAX gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an PTMAX gene, (8) a non-wild type level of an

20 PTMAX-protein, (9) allelic loss of an PTMAX gene, and (10) inappropriate post-translational modification of an PTMAX-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an PTMAX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional

25 means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*,

Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the PTMAX-gene (see Abravaya *et al.* (1995) *Nucl Acids Res* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an PTMAX gene under conditions such that hybridization and amplification of the PTMAX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a PTMAX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in PTMAX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7: 244-255;

Kozal *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in PTMAX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* above. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the PTMAX gene and detect mutations by comparing the sequence of the sample PTMAX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (1977) *PNAS* 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.*, (1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publ. No. WO 94/16101; Cohen *et al.* (1996) *Adv Chromatogr* 36:127-162; and Griffin *et al.* (1993) *Appl Biochem Biotechnol* 38:147-159).

Other methods for detecting mutations in the PTMAX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type PTMAX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest

mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* (1988) *Proc Natl Acad Sci USA* 85:4397; Saleeba *et al* (1992) *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

5 In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in PTMAX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at  
10 G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a PTMAX sequence, *e.g.*, a wild-type PTMAX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in PTMAX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl Acad Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA  
20 fragments of sample and control PTMAX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is  
25 more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel

electrophoresis (DGGE) (Myers *et al* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al* (1992) *Mol Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc Natl Acad Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein,

which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an PTMAX gene.

Furthermore, any cell type or tissue, preferably thymus tissue, in which PTMAX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

### Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on PTMAX activity (*e.g.*, PTMAX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (*e.g.*, cancer or immune disorders associated with aberrant PTMAX activity. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of PTMAX protein, expression of PTMAX nucleic acid, or mutation content of PTMAX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, *Clin Exp Pharmacol Physiol*, 1996, 23:983-985 and Linder, *Clin Chem*, 1997, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a

common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of PTMAX protein, expression of PTMAX nucleic acid, or mutation content of PTMAX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an PTMAX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

## Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of PTMAX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase PTMAX gene expression, protein levels, or upregulate PTMAX activity, can be monitored in clinical trails of subjects exhibiting decreased PTMAX gene expression, protein levels, or downregulated PTMAX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease PTMAX gene expression, protein levels, or downregulate PTMAX activity, can be monitored in clinical trails of subjects exhibiting increased PTMAX gene expression, protein levels, or upregulated PTMAX activity. In such clinical trials, the expression or activity of PTMAX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

For example, and not by way of limitation, genes, including PTMAX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates PTMAX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of PTMAX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of PTMAX or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the



screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an PTMAX protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the PTMAX protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the PTMAX protein, mRNA, or genomic DNA in the pre-administration sample with the PTMAX protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of PTMAX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of PTMAX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

### Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant PTMAX expression or activity. For example, PTMA 1-6, 9 and 10 will be useful for both prophylactic and therapeutic methods of treating various cancers, viral diseases, and immune deficiency disorders. As a further example, PTMA 7 will be useful for both prophylactic and therapeutic methods of treating various cancers, coronary artery disease, arthritis, diabetic retinopathy, autoimmune diseases, and immune deficiency disorders. As a further example, PTMA 8 will be useful for both prophylactic and therapeutic methods of treating neurological diseases, psychiatric disorders, and inflammatory diseases.

### Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be

utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (see, *e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

### Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant PTMAX expression or activity, by administering to the subject an agent that modulates PTMAX expression or at least one PTMAX activity. Subjects at risk for a disease that is caused or contributed to by aberrant PTMAX expression or activity can

be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the PTMAX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of PTMAX aberrancy, for example, an PTMAX agonist or PTMAX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

### Therapeutic Methods

Another aspect of the invention pertains to methods of modulating PTMAX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of PTMAX protein activity associated with the cell. An agent that modulates PTMAX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an PTMAX protein, a peptide, an PTMAX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more PTMAX protein activity. Examples of such stimulatory agents include active PTMAX protein and a nucleic acid molecule encoding PTMAX that has been introduced into the cell. In another embodiment, the agent inhibits one or more PTMAX protein activity. Examples of such inhibitory agents include antisense PTMAX nucleic acid molecules and anti-PTMAX antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a PTMAX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) PTMAX expression or activity. In another embodiment, the method involves administering an PTMAX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant PTMAX expression or activity.

Stimulation of PTMAX activity is desirable in situations in which PTMAX is abnormally downregulated and/or in which increased PTMAX activity is likely to have a beneficial effect.

One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (*e.g.*, preclampsia).

### **Determination of the Biological Effect of the Therapeutic**

5           In various embodiments of the present invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

10           In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

### **Malignancies**

15           Therapeutics of the present invention may be useful in the therapeutic or prophylactic treatment of diseases or disorders that are associated with cell hyperproliferation and/or loss of control of cell proliferation (*e.g.*, cancers, malignancies and tumors). For a review of such hyperproliferation disorders, see *e.g.*, Fishman, *et al.*, 1985. MEDICINE, 2nd ed., J.B. Lippincott Co., Philadelphia, PA.

20           Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing malignancies and related disorders. Such assays include, but are not limited to, *in vitro* assays utilizing transformed cells or cells derived from the patient's tumor, as well as *in vivo* assays using animal models of cancer or malignancies. Potentially effective Therapeutics are those that, for example, inhibit the proliferation of  
25           tumor-derived or transformed cells in culture or cause a regression of tumors in animal models, in comparison to the controls.

In the practice of the present invention, once a malignancy or cancer has been shown to be amenable to treatment by modulating (*i.e.*, inhibiting, antagonizing or agonizing) activity, that cancer or malignancy may subsequently be treated or prevented by the administration of a Therapeutic that serves to modulate protein function.

## 5 Premalignant conditions

The Therapeutics of the present invention that are effective in the therapeutic or prophylactic treatment of cancer or malignancies may also be administered for the treatment of pre-malignant conditions and/or to prevent the progression of a pre-malignancy to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia or, most particularly, dysplasia has occurred. For a review of such abnormal cell growth see *e.g.*, Robbins & Angell, 1976. BASIC PATHOLOGY, 2nd ed., W.B. Saunders Co., Philadelphia, PA.

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in its structure or function. For example, it has been demonstrated that endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of mature or fully differentiated cell substitutes for another type of mature cell. Metaplasia may occur in epithelial or connective tissue cells. Dysplasia is generally considered a precursor of cancer, and is found mainly in the epithelia. Dysplasia is the most disorderly form of non-neoplastic cell growth, and involves a loss in individual cell uniformity and in the architectural orientation of cells. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively, or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed or malignant phenotype displayed either *in vivo* or *in vitro* within a cell sample derived from a patient, is indicative of the desirability of prophylactic/therapeutic administration of a Therapeutic that possesses the ability to modulate activity of An aforementioned protein.

Characteristics of a transformed phenotype include, but are not limited to: (i) morphological changes; (ii) looser substratum attachment; (iii) loss of cell-to-cell contact inhibition; (iv) loss of anchorage dependence; (v) protease release; (vi) increased sugar transport; (vii) decreased serum requirement; (viii) expression of fetal antigens, (ix) disappearance of the 250 kDal cell-surface protein, and the like. See *e.g.*, Richards, *et al.*, 1986. MOLECULAR PATHOLOGY, W.B. Saunders Co., Philadelphia, PA.

In a specific embodiment of the present invention, a patient that exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: (i) a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome (*bcr/abl*) for chronic myelogenous leukemia and t(14;18) for follicular lymphoma, etc.); (ii) familial polyposis or Gardner's syndrome (possible forerunners of colon cancer); (iii) monoclonal gammopathy of undetermined significance (a possible precursor of multiple myeloma) and (iv) a first degree kinship with persons having a cancer or pre-cancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, medullary thyroid carcinoma with amyloid production and pheochromocytoma, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia and Bloom's syndrome).

In another embodiment, a Therapeutic of the present invention is administered to a human patient to prevent the progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

### **Hyperproliferative and dysproliferative disorders**

In one embodiment of the present invention, a Therapeutic is administered in the therapeutic or prophylactic treatment of hyperproliferative or benign dysproliferative disorders. The efficacy in treating or preventing hyperproliferative diseases or disorders of a Therapeutic of the present invention may be assayed by any method known within the art. Such assays include *in vitro* cell proliferation assays, *in vitro* or *in vivo* assays using animal models of

hyperproliferative diseases or disorders, or the like. Potentially effective Therapeutics may, for example, promote cell proliferation in culture or cause growth or cell proliferation in animal models in comparison to controls.

Specific embodiments of the present invention are directed to the treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes); treatment of keloid (hypertrophic scar) formation causing disfiguring of the skin in which the scarring process interferes with normal renewal; psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination); benign tumors; fibrocystic conditions and tissue hypertrophy (e.g., benign prostatic hypertrophy).

#### Neurodegenerative disorders

PTMAX protein have been implicated in the deregulation of cellular maturation and apoptosis, which are both characteristic of neurodegenerative disease. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) activity of an aforementioned protein, may be effective in treating or preventing neurodegenerative disease. Therapeutics of the present invention that modulate the activity of an aforementioned protein involved in neurodegenerative disorders can be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include *in vitro* assays for regulated cell maturation or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders, or any of the assays described below. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation activity, that neurodegenerative disease or disorder can be treated or prevented by administration of a Therapeutic that modulates activity. Such diseases include all degenerative disorders involved with aging, especially osteoarthritis and neurodegenerative disorders.

## Disorders related to organ transplantation

PTMAX has been implicated in disorders related to organ transplantation, in particular but not limited to organ rejection. Therapeutics of the invention, particularly those that modulate (or supply) activity, may be effective in treating or preventing diseases or disorders related to organ transplantation. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of an aforementioned protein) can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders related to organ transplantation. Such assays include *in vitro* assays for using cell culture models as described below, or *in vivo* assays using animal models of diseases and disorders related to organ transplantation, see *e.g.*, below. Potentially effective Therapeutics, for example but not by way of limitation, reduce immune rejection responses in animal models in comparison to controls.

Accordingly, once diseases and disorders related to organ transplantation are shown to be amenable to treatment by modulation of activity, such diseases or disorders can be treated or prevented by administration of a Therapeutic that modulates activity.

## Cytokine and Cell Proliferation/Differentiation Activity

A PTMAX protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+ ), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods: Assays for T-cell or thymocyte proliferation include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 3 and Chapter 7); Takai *et al.*, *J Immunol*



137:3494-3500, 1986; Bertagnolli *et al.*, *J Immunol* 145:1706-1712, 1990; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Bertagnolli, *et al.*, *J Immunol* 149:3778-3783, 1992; Bowman *et al.*, *J Immunol* 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described by Kruisbeek and Shevach, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1, pp. 3.12.1-14, John Wiley and Sons, Toronto 1994; and by Schreiber, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan eds. Vol 1 pp. 6.8.1-8, John Wiley and Sons, Toronto 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described by Bottomly *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto 1991; deVries *et al.*, *J Exp Med* 173:1205-1211, 1991; Moreau *et al.*, *Nature* 336:690-692, 1988; Greenberger *et al.*, *Proc Natl Acad Sci U.S.A.* 80:2931-2938, 1983; Nordan, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.6.1-5, John Wiley and Sons, Toronto 1991; Smith *et al.*, *Proc Natl Acad Sci U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11-Bennett, *et al.* In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto 1991; Ciarletta, *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Greene Publishing Associates and Wiley-Interscience (Chapter 3Chapter 6, Chapter 7); Weinberger *et al.*, *Proc Natl Acad Sci USA* 77:6091-6095, 1980; Weinberger *et al.*, *Eur J Immun* 11:405-411, 1981; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988.

## Immune Stimulating or Suppressing Activity

A PTMAX protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), *e.g.*, in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by vital (*e.g.*, HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by vital, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, *Leishmania* species., malaria species. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T

cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or energy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon re-exposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to energize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*,

Science 257:789-792 (1992) and Turka *et al.*, Proc Natl Acad Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

5           Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto-antibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of  
10 T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto-antibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 840-856).

20           Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic vital  
25 diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

          Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of

a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-vital immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I chain protein and  $\beta$  2 microglobulin protein or an MHC class II  $\alpha$  chain protein and an MHC class II chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant

chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

5           The activity of a protein of the invention may, among other means, be measured by the following methods: Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 135:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 135:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Bowman *et al.*, *J Virology* 61:1992-1998; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Brown *et al.*, *J Immunol* 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, *J Immunol* 144:3028-3033, 1990; and Mond and Brunswick In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, (eds.) Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *J Immunol* 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery *et al.*, *J Immunol* 134:536-544, 1995; Inaba *et al.*, *J Exp Med* 173:549-559, 1991; Macatonia *et al.*, *J Immunol* 154:5071-5079, 1995; Porgador *et al.*, *J Exp Med* 182:255-260, 1995; Nair *et al.*,

*J Virol* 67:4062-4069, 1993; Huang *et al.*, *Science* 264:961-965, 1994; Macatonia *et al.*, *J Exp Med* 169:1255-1264, 1989; Bhardwaj *et al.*, *J Clin Investig* 94:797-807, 1994; and Inaba *et al.*, *J Exp Med* 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz *et al.*, *Cytometry* 13:795-808, 1992; Gorczyca *et al.*, *Leukemia* 7:659-670, 1993; Gorczyca *et al.*, *Cancer Res* 53:1945-1951, 1993; Itoh *et al.*, *Cell* 66:233-243, 1991; Zacharchuk, *J Immunol* 145:4037-4045, 1990; Zamai *et al.*, *Cytometry* 14:891-897, 1993; Gorczyca *et al.*, *Internat J Oncol* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica *et al.*, *Blood* 84:111-117, 1994; Fine *et al.*, *Cell Immunol* 155: 111-122, 1994; Galy *et al.*, *Blood* 85:2770-2778, 1995; Toki *et al.*, *Proc Nat Acad Sci USA* 88:7548-7551, 1991.

### **Hematopoiesis Regulating Activity**

A PTMAX protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, *e.g.* in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (*i.e.*, traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of

hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (*i.e.*, in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson *et al.* *Cellular Biology* 15:141-151, 1995; Keller *et al.*, *Mol. Cell. Biol.* 13:473-486, 1993; McClanahan *et al.*, *Blood* 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* (eds.) Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama *et al.*, *Proc Natl Acad Sci USA* 89:5907-5911, 1992; McNiece and Briddeli, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* (eds.) Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben *et al.*, *Exp Hematol* 22:353-359, 1994; Ploemacher, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Spooncer *et al.*, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.*, (eds.) Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Sutherland, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.*, (eds.) Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.



## Tissue Growth Activity

A PTMAX protein of the present invention also may have utility in compositions used for nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement.

5           The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral  
10   nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for  
20   generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic  
25   activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, EPIDERMAL WOUND HEALING, pp. 71-112 (Maibach and Rovee, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Menz, *J. Invest. Dermatol* 71:382-84 (1978).

#### **Chemotactic/Chemokinetic Activity**

A protein of the present invention may have chemotactic or chemokinetic activity (*e.g.*, act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Coligan *et al.*, eds. (Chapter 6.12, MEASUREMENT OF ALPHA AND BETA CHEMOKINES 6.12.1-6.12.28); Taub *et al. J Clin Invest* 95:1370-1376, 1995; Lind *et al. APMIS* 103:140-146, 1995; Muller *et al., Eur J Immunol* 25: 1744-1748; Gruberet *al. J Immunol* 152:5860-5867, 1994; Johnston *et al., J Immunol* 153: 1762-1768, 1994.

### Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell—cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan, *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions

7.28.1-7.28.22), Takai *et al.*, *Proc Natl Acad Sci USA* 84:6864-6868, 1987; Bierer *et al.*, *J. Exp. Med.* 168:1145-1156, 1988; Rosenstein *et al.*, *J. Exp. Med.* 169:149-160 1989; Stoltenborg *et al.*, *J Immunol Methods* 175:59-68, 1994; Stitt *et al.*, *Cell* 80:661-670, 1995.

### **Anti-Inflammatory Activity**

5 Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell—cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production  
10 of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or hypersensitivity to an antigenic substance or material.

### **Tumor Inhibition Activity**

20 In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor  
25 growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

## EXAMPLES

### Example 1. Radiation Hybrid Mapping for Various Clones

#### Radiation Hybrid Mapping Provides the Chromosomal Location of Clones.

5

Radiation hybrid mapping using human chromosome markers was carried out for many of the clones described in the present invention. The procedure used to obtain these results is analogous to that described in Steen, RG et al. (A High-Density Integrated Genetic Linkage and Radiation Hybrid Map of the Laboratory Rat, Genome Research 1999 (Published Online on May 21, 1999) Vol. 9, AP1-AP8, 1999). A panel of 93 cell clones containing randomized radiation-

10 induced human chromosomal fragments was screened in 96 well plates using PCR primers designed to identify the sought clones in a unique fashion. Table 3 provides the results obtained for clones AC010784-1 and AC010175\_A.0.1.

**Table 3. Chromosomal mapping from radiation hybrid results.**

Clone	Chromosome	Distance from Marker, cR	Distance from Marker, cR
AC010784-1	4	WI-4767, 8.4cR	WI-5565, 0.0cR
AC010175_A.0.1	12	D12S358, 4.2cR	AFMA184ZC1, 2.5 cR

### Example 2. Quantitative expression analysis of PTMAX nucleic acids

The quantitative expression of various clones was assessed in about 40 normal and about 54 tumor samples (the samples are identified in the Tables below) by real time quantitative PCR (TAQMAN®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System.

20

First, 96 RNA samples were normalized to  $\beta$ -actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; cat # N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48°C. cDNA (5 ul) was then transferred to a separate plate for the TAQMAN® reaction using  $\beta$ -actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; cat. #'s 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; cat # 4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for  $\beta$ -actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their  $\beta$ -actin /GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; cat. # 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) using the sequence of the subject clone as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature ( $T_m$ ) range = 58°-60° C, primer optimal  $T_m$  = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe  $T_m$  must be 10° C greater than primer  $T_m$ , amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (PROTHYAX-specific and another gene-specific probe multiplexed with the PROTHYAX probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl<sub>2</sub>, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/ l RNase inhibitor, and 0.25 U/ l reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

10

**A. Clone Identification No: AC010175 (PTMA 6)**

**Probe Name: Ag165**

Primers	Sequences	SEQ ID NO
Forward	5'-ATGTCAGACGCAGCCGTAGA-3'	21
Probe	TET-5'-ACCAGCTCCGAAATCACCACCGAG-3'-TAMRA	22
Reverse	5'-CTTCCACAACCTCCTTCTTCTCCT-3'	23

Tissue_Name	% Rel. Expr.	Tissue_Name	% Rel. Expr.
Endothelial cells	11.7	Kidney (fetal)	51.1
Endothelial cells (treated)	15.2	Renal ca. 786-0	12.9
Pancreas	24.2	Renal ca. A498	4.7
Pancreatic ca. CAPAN 2	34.6	Renal ca. RXF 393	4.7

Adipose	37.6	Renal ca. ACHN	13.6
Adrenal gland	25.9	Renal ca. UO-31	6.0
Thyroid	34.9	Renal ca. TK-10	13.9
Salivary gland	16.8	Liver	26.8
Pituitary gland	11.1	Liver (fetal)	15.7
Brain (fetal)	17.3	Liver ca. (hepatoblast) HepG2	5.1
Brain (whole)	36.1	Lung	13.9
Brain (amygdala)	14.7	Lung (fetal)	29.5
Brain (cerebellum)	50.4	Lung ca. (small cell) LX-1	24.2
Brain (hippocampus)	23.3	Lung ca. (small cell) NCI-H69	13.2
Brain (substantia nigra)	33.2	Lung ca. (s.cell var.) SHP-77	0.0
Brain (thalamus)	21.8	Lung ca. (large cell) NCI-H460	0.0
Brain (hypothalamus)	8.6	Lung ca. (non-sm. cell) A549	9.6
Spinal cord	16.0	Lung ca. (non-s.cell) NCI-H23	13.3
CNS ca. (glio/astro) U87-MG	7.4	Lung ca (non-s.cell) HOP-62	7.1
CNS ca. (glio/astro) U-118-MG	8.4	Lung ca. (non-s.cl) NCI-H522	28.5
CNS ca. (astro) SW1783	6.6	Lung ca. (squam.) SW 900	37.6
CNS ca.* (neuro; met ) SK-N-AS	24.3	Lung ca. (squam.) NCI-H596	27.6
CNS ca. (astro) SF-539	7.3	Mammary gland	27.4
CNS ca. (astro) SNB-75	9.0	Breast ca.* (pl. effusion) MCF-7	50.4
CNS ca. (glio) SNB-19	13.9	Breast ca.* (pl.ef) MDA-MB-231	11.5
CNS ca. (glio) U251	7.4	Breast ca.* (pl. effusion) T47D	27.4
CNS ca. (glio) SF-295	7.1	Breast ca. BT-549	0.0
Heart	10.1	Breast ca. MDA-N	23.0
Skeletal muscle	3.0	Ovary	23.5
Bone marrow	24.2	Ovarian ca. OVCAR-3	8.8
Thymus	74.2	Ovarian ca. OVCAR-4	7.3



Spleen	22.9	Ovarian ca. OVCAR-5	17.4
Lymph node	37.6	Ovarian ca. OVCAR-8	23.5
Colon (ascending)	28.1	Ovarian ca. IGROV-1	8.4
Stomach	19.6	Ovarian ca.* (ascites) SK-OV-3	19.8
Small intestine	21.6	Uterus	17.1
Colon ca. SW480	9.2	Placenta	34.9
Colon ca.* (SW480 met)SW620	17.7	Prostate	21.6
Colon ca. HT29	27.6	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	26.6
Colon ca. CaCo-2	17.6	Melanoma Hs688(A).T	8.9
Colon ca. HCT-15	19.9	Melanoma* (met) Hs688(B).T	5.3
Colon ca. HCC-2998	20.6	Melanoma UACC-62	1.7
Gastric ca.* (liver met) NCI-N87	42.9	Melanoma M14	19.2
Bladder	28.1	Melanoma LOX IMVI	100.0
Trachea	31.0	Melanoma* (met) SK-MEL-5	13.6
Kidney	18.8	Melanoma SK-MEL-28	19.3

ca. = carcinoma

\* = established from metastasis

met = metastasis

s cell var= small cell variant

non-s = non-sm =non-small

squam = squamous

pl. eff = pl effusion = pleural effusion

glio = glioma

astro = astrocytoma

neuro = neuroblastoma

It is seen from the Table above that clone AC010175 is expressed in most normal and cancer cells assayed. It is especially prominent in Melanoma LOX IMVI and thymus.

**B. Clone Identification No: AC009485\_A (PTMA 1)****Probe Name: Ag184**

Primers	Sequences	SEQ ID NO
Forward	5'-AGAGGAAGCTGAGTCTGCTACAGG-3'	24
Probe	5'-CCTCATCATCTTCAGCTGCCCCGCTT-3'- TAMRA	25
Reverse	5'-TCTGCTTCTTGGTATCGACATCAT-3'	26

Tissue_Name	% Relative Expr.	Tissue_Name	% Relative Expr.
Endothelial cells	31.6	Kidney (fetal)	76.8
Endothelial cells (treated)	36.6	Renal ca. 786-0	37.6
Pancreas	68.8	Renal ca. A498	20.3
Pancreatic ca. CAPAN 2	79.0	Renal ca. RXF 393	29.7
Adipose	69.3	Renal ca. ACHN	41.8
Adrenal gland	47.0	Renal ca. UO-31	28.9
Thyroid	87.1	Renal ca. TK-10	60.3
Salivary gland	26.8	Liver	55.1
Pituitary gland	48.6	Liver (fetal)	38.4
Brain (fetal)	42.0	Liver ca. (hepatoblast) HepG2	24.8
Brain (whole)	53.2	Lung	31.4
Brain (amygdala)	34.6	Lung (fetal)	77.4
Brain (cerebellum)	59.1	Lung ca. (small cell) LX-1	57.8

Brain (hippocampus)	39.2	Lung ca. (small cell) NCI-H69	33.9
Brain (substantia nigra)	76.8	Lung ca. (s.cell var.) SHP-77	82.9
Brain (thalamus)	46.7	Lung ca. (large cell) NCI-H460	62.0
Brain (hypothalamus)	43.5	Lung ca. (non-sm. cell) A549	33.9
Spinal cord	59.9	Lung ca. (non-s.cell) NCI-H23	36.1
CNS ca. (glio/astro) U87-MG	28.3	Lung ca (non-s.cell) HOP-62	31.4
CNS ca. (glio/astro) U-118-MG	39.5	Lung ca. (non-s.cl) NCI-H522	69.7
CNS ca. (astro) SW1783	12.4	Lung ca. (squam.) SW 900	62.4
CNS ca.* (neuro; met) SK-N-AS	76.3	Lung ca. (squam.) NCI-H596	75.8
CNS ca. (astro) SF-539	16.7	Mammary gland	71.2
CNS ca. (astro) SNB-75	25.2	Breast ca.* (pl. effusion) MCF-7	68.8
CNS ca. (glio) SNB-19	45.7	Breast ca.* (pl.ef) MDA-MB-231	27.0
CNS ca. (glio) U251	20.6	Breast ca.* (pl. effusion) T47D	49.0
CNS ca. (glio) SF-295	19.6	Breast ca. BT-549	73.7
Heart	26.4	Breast ca. MDA-N	60.7
Skeletal muscle	22.4	Ovary	59.1
Bone marrow	65.1	Ovarian ca. OVCAR-3	43.2
Thymus	100.0	Ovarian ca. OVCAR-4	37.4
Spleen	76.3	Ovarian ca. OVCAR-5	75.3
Lymph node	81.2	Ovarian ca. OVCAR-8	59.1
Colon (ascending)	55.5	Ovarian ca. IGROV-1	27.0
Stomach	60.3	Ovarian ca.* (ascites) SK-OV-3	67.8
Small intestine	57.8	Uterus	58.2
Colon ca. SW480	48.0	Placenta	65.5
Colon ca.* (SW480 met) SW620	66.4	Prostate	50.0
Colon ca. HT29	88.3	Prostate ca.* (bone met) PC-3	66.9
Colon ca. HCT-116	98.6	Testis	77.4

Colon ca. CaCo-2	39.5	Melanoma Hs688(A).T	30.8
Colon ca. HCT-15	51.8	Melanoma* (met) Hs688(B).T	8.9
Colon ca. HCC-2998	50.0	Melanoma UACC-62	3.2
Gastric ca.* (liver met) NCI-N87	65.5	Melanoma M14	27.4
Bladder	63.3	Melanoma LOX IMVI	94.0
Trachea	75.3	Melanoma* (met) SK-MEL-5	47.0
Kidney	51.1	Melanoma SK-MEL-28	47.0

As seen in the Table above, clone AC009485\_A is highly expressed in most normal and cancer cell lines examined, especially in thymus and Melanoma LOX IMVI.

**C. Clone Identification No: AC009533\_A (PTMA 4)**

**Probe Name: Ag185**

Primers	Sequences	SEQ ID NO
Forward	5'-AGATGTCAGACGCAGCCGTA-3'	27
Probe	TET-5'-CAGCTCCGAAATCACCACCGAGGAC-3'- TAMRA	28
Reverse	5'-TCCACAACCTTCCTTCTTCTCCTTT-3'	29

10

Tissue_Name	% Rel.	% Rel.	Tissue_Name	% Rel.	% Rel.
	Expr.	Expr.		Expr.	Expr.

	tm381t	tm336t		tm381t	tm336t
Endothelial cells	4.3	1.5	Kidney (fetal)	37.6	39.0
Endothelial cells (treated)	15.4	3.3	Renal ca. 786-0	8.8	2.9
Pancreas	20.2	20.6	Renal ca. A498	0.9	0.6
Pancreatic ca. CAPAN 2	22.9	22.2	Renal ca. RXF 393	1.2	0.6
Adipose	44.4	55.9	Renal ca. ACHN	2.8	2.6
Adrenal gland	6.7	2.5	Renal ca. UO-31	0.4	0.6
Thyroid	30.4	51.1	Renal ca. TK-10	4.6	8.1
Salivary gland	5.2	2.2	Liver	21.9	11.6
Pituitary gland	3.6	8.4	Liver (fetal)	4.7	6.0
Brain (fetal)	2.4	4.1	Liver ca. (hepatoblast) HepG2	1.5	0.6
Brain (whole)	14.1	11.7	Lung	10.7	16.0
Brain (amygdala)	2.3	3.5	Lung (fetal)	13.2	44.4
Brain (cerebellum)	37.4	28.5	Lung ca. (small cell) LX-1	27.4	27.9
Brain (hippocampus)	6.8	8.3	Lung ca. (small cell) NCI-H69	5.3	3.3
Brain (substantia nigra)	22.4	15.0	Lung ca. (s.cell var.) SHP-77	59.5	65.5
Brain (thalamus)	12.9	12.9	Lung ca. (large cell) NCI-H460	14.2	25.4
Brain (hypothalamus)	1.9	8.6	Lung ca. (non-sm. cell) A549	3.3	5.0
Spinal cord	10.1	4.2	Lung ca. (non-s.cell) NCI-H23	9.9	6.8
CNS ca. (glio/astro) U87-MG	3.0	0.6	Lung ca (non-s.cell) HOP-62	0.8	0.6
CNS ca. (glio/astro) U-118-MG	2.5	1.9	Lung ca. (non-s.cl) NCI-H522	19.2	26.8
CNS ca. (astro) SW1783	1.2	0.6	Lung ca. (squam.) SW 900	27.0	33.9
CNS ca.* (neuro; met ) SK-N-AS	29.1	33.9	Lung ca. (squam.) NCI-H596	23.8	37.4
CNS ca. (astro) SF-539	1.0	0.6	Mammary gland	25.4	34.4
CNS ca. (astro) SNB-75	1.4	0.6	Breast ca.* (pl. effusion) MCF-7	50.0	66.0
CNS ca. (glio) SNB-19	4.3	5.9	Breast ca.* (pl.ef) MDA-MB-231	3.1	0.8

CNS ca. (glio) U251	0.7	0.8	Breast ca.* (pl. effusion) T47D	13.7	12.2
CNS ca. (glio) SF-295	0.5	1.5	Breast ca. BT-549	40.1	38.7
Heart	3.6	1.3	Breast ca. MDA-N	13.1	25.4
Skeletal muscle	0.0	0.6	Ovary	33.2	23.0
Bone marrow	13.3	26.4	Ovarian ca. OVCAR-3	4.4	4.4
Thymus	66.0	100.0	Ovarian ca. OVCAR-4	2.4	2.4
Spleen	14.7	25.0	Ovarian ca. OVCAR-5	12.2	27.0
Lymph node	27.6	46.7	Ovarian ca. OVCAR-8	12.3	17.2
Colon (ascending)	29.3	27.4	Ovarian ca. IGROV-1	1.3	0.6
Stomach	12.9	19.5	Ovarian ca.* (ascites) SK-OV-3	11.7	21.6
Small intestine	18.4	25.2	Uterus	9.7	11.6
Colon ca. SW480	3.1	1.4	Placenta	33.5	33.7
Colon ca.* (SW480 met)SW620	11.8	17.1	Prostate	14.6	15.5
Colon ca. HT29	14.1	40.1	Prostate ca.* (bone met)PC-3	20.0	16.2
Colon ca. HCT-116	64.6	82.9	Testis	22.2	22.5
Colon ca. CaCo-2	7.6	7.8	Melanoma Hs688(A).T	1.1	0.6
Colon ca. HCT-15	13.2	13.5	Melanoma* (met) Hs688(B).T	0.1	0.6
Colon ca. HCC-2998	9.9	5.1	Melanoma UACC-62	0.0	0.6
Gastric ca.* (liver met) NCI-N87	26.2	40.3	Melanoma M14	6.8	8.4
Bladder	18.7	28.7	Melanoma LOX IMVI	100.0	76.8
Trachea	27.6	33.0	Melanoma* (met) SK-MEL-5	3.3	10.0
Kidney	10.7	7.5	Melanoma SK-MEL-28	9.5	6.0

The Table above shows that clone AC009533\_A is highly expressed in many normal and cancer cell lines. It is highly expressed especially in melanoma LOX IMVI, breast ca.\* (pl. effusion) MCF-7, lung ca. (s.cell var.) SHP-77, and colon ca. HCT-116, as well as in normal thymus cells.

**D. Clone Identification No: AL121585\_A (PTMA 5)****Probe Name: Ag1091**

Primers	Sequences	SEQ ID NO
Forward	5'-TGCCTATACCAAGAAGCAGAAG-3'	30
Probe	FAM-5'-CCAACAAGGATGACTAGACAGCAAAA-3'- TAMRA	31
Reverse	5'-TGAATAGGTCACCCTCCTAACA-3'	32

Tissue_Name	% Relative Expr.	Tissue_Name	% Relative Expr.
Endothelial cells	0.0	Kidney (fetal)	10.4
Endothelial cells (treated)	0.0	Renal ca. 786-0	0.0
Pancreas	8.7	Renal ca. A498	0.0
Pancreatic ca. CAPAN 2	27.7	Renal ca. RXF 393	0.0
Adipose	100.0	Renal ca. ACHN	0.0
Adrenal Gland (new lot*)	0.0	Renal ca. UO-31	0.0
Thyroid	2.0	Renal ca. TK-10	2.7
Salivary gland	47.0	Liver	0.0
Pituitary gland	4.5	Liver (fetal)	0.0
Brain (fetal)	2.5	Liver ca. (hepatoblast) HepG2	0.0
Brain (whole)	0.8	Lung	7.0

Brain (amygdala)	0.4	Lung (fetal)	4.8
Brain (cerebellum)	0.0	Lung ca. (small cell) LX-1	2.1
Brain (hippocampus)	0.8	Lung ca. (small cell) NCI-H69	94.6
Brain (thalamus)	0.0	Lung ca. (s.cell var.) SHP-77	0.0
Cerebral Cortex	0.9	Lung ca. (large cell)NCI-H460	0.0
Spinal cord	0.0	Lung ca. (non-sm. cell) A549	0.6
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca (non-s.cell) HOP-62	0.0
CNS ca. (astro) SW1783	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
CNS ca.* (neuro; met ) SK-N-AS	0.0	Lung ca. (squam.) SW 900	37.4
CNS ca. (astro) SF-539	0.0	Lung ca. (squam.) NCI-H596	28.3
CNS ca. (astro) SNB-75	0.0	Mammary gland	10.2
CNS ca. (glio) SNB-19	0.0	Breast ca.* (pl. effusion) MCF-7	2.7
CNS ca. (glio) U251	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
CNS ca. (glio) SF-295	0.0	Breast ca.* (pl. effusion) T47D	41.2
Heart	0.0	Breast ca. BT-549	0.0
Skeletal Muscle (new lot*)	0.0	Breast ca. MDA-N	0.0
Bone marrow	0.0	Ovary	0.0
Thymus	0.0	Ovarian ca. OVCAR-3	17.2
Spleen	0.0	Ovarian ca. OVCAR-4	7.9
Lymph node	0.0	Ovarian ca. OVCAR-5	3.5
Colorectal	3.7	Ovarian ca. OVCAR-8	0.0
Stomach	88.3	Ovarian ca. IGROV-1	0.0
Small intestine	30.8	Ovarian ca.* (ascites) SK-OV-3	2.7
Colon ca. SW480	0.2	Uterus	1.4
Colon ca.* (SW480 met)SW620	0.0	Placenta	1.4
Colon ca. HT29	1.5	Prostate	81.8



Colon ca. HCT-116	0.0	Prostate ca.* (bone met)PC-3	0.0
Colon ca. CaCo-2	9.7	Testis	3.5
83219 CC Well to Mod Diff (ODO3866)	3.4	Melanoma Hs688(A).T	0.0
Colon ca. HCC-2998	93.3	Melanoma* (met) Hs688(B).T	0.0
Gastric ca.* (liver met) NCI-N87	64.6	Melanoma UACC-62	0.0
Bladder	20.2	Melanoma M14	1.5
Trachea	14.7	Melanoma LOX IMVI	0.0
Kidney	19.5	Melanoma* (met) SK-MEL-5	0.0

It is seen from the above Table that clone AL121585\_A is highly expressed in certain cell lines and weakly or not at all in many others. It is highly expressed in normal prostate, stomach and adipose, and in breast ca.\* (pl. effusion) T47D, lung ca. (small cell) NCI-H69, gastric ca.\* (liver met) NCI-N87 and colon ca. HCC-2998.

#### OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims